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Genetic Engineering and Biotechnology Monitor

Issue No. 31 November 1990

N.B. This issue carries an article on the concepts of risk assessment, which is based on a presentation made at the Fourth Meeting of the Informal UNIDO/WHO/UNEP Working Group on Biotechnology Safety, by Dr. A. Lazen, Director of Program Operations, Commission on Life Sciences, National Research Council, US National Academy of Sciences.

This publication is distributed free of charge

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A. POLICY NEWS AND OTHER EVENTS

UNIDO News

ICGEB news

A reorientation of the rolling five-year programme (July 1990 to June 1995) of the International Centre for Genetic Engineering and Biotechnology was approved in Vienna at the 14th session of the Centre's Preparatory Committee (31 January-2 February).

During 1994-1995, long-term post-doctoral training will be increased by 20 additional years for both the New Delhi and Trieste laboratories, making a total of 48 years each.

In 1990, 50 per cent more training courses will be offered at the Centre than last year. Likewise, long-term fellowships are expected to increase to 30 compared with the current 16 that have been awarded so far.

* * *

India reaffirmed its support of ICGEB in an exchange of letters on 28 February between Indian Permanent Representative, Peter Sinai, and Director-General, Domingo L. Siazon, Jr.

This extends the former agreement on basic terms and conditions governing the UNIDO ICGEB project from January 1990 to March 1991. During this period, the Five-Year Programme (1989-1994) will be under way at the New Delhi and Trieste laboratories.

* * *

The Bombay pharmaceutical firm Wockhardt Ltd. became the first private company to make a major contribution to ICGEB. This came in the form of a research grant under an agreement signed on 15 March by Wockhardt Managing Director, Habil F. Khorakiwala, and Director-General, Domingo L. Saizon, Jr.

According to the agreement, Wockhardt Ltd. will contribute up to Rs. 50 million, equivalent to more than $3 million, for research at the Centre's New Delhi laboratory in insulin, tissue plasminogen activator, erythropoietin, hepatitis vaccine and another mutually-agreed product during the next five years.

Wockhardt, which is committed to development and commercialization of these products, will undertake their manufacturing. On behalf of ICGEB, UNIDO will receive royalties from sales in India and abroad. The Organization will carry out these activities in co-operation with the Government of India.

Wockhardt is the recipient of the 1988 Indian National Award for R+D Efforts in Industry.

United Nations and other organizations' news

FAO and plant genetic resources

The FAO Commission on Plant Genetic Resources has requested FAO to draft a Code of Conduct for Biotechnology as it affects conservation and use of plant genetic resources. The Code of Co duct will take into consideration the technical, economical, social, ethical, political, legal and environmental implications that the new biotechnology may/will have in developed and developing countries. (Extracted from ABA Bulletin, Vol. 5, No. 2, April 1990)

WHO finds new director for AIDS programme

The World Health Organization has appointed Michael Merson as the new director of its Global Programme on AIDS. Merson, who was formerly director of the WHO's Diarrhoeal Disease Control programme, succeeds Jonathan Mann who resigned in March. Merson's appointment comes shortly after the US and other countries that donate money to the WHO's Global Programme on AIDS announced that they would not increase their funding to the programme next year. The programme spent $75 million in 1989, and expects to spend $91 million in 1990. Next year, the donor countries say their total funds will not exceed $75 million. (Source: New Scientist, 19 May 1990)

WHO to speed up work on drugs for tropical diseases

Basic research into tropical diseases, now infecting almost half a billion people world-wide, faces serious setbacks unless developed countries agree to increase funding. The warning comes in a new report by the World Health Organization. It estimates that one person in 10 suffers from one or more tropical diseases (see table), yet they account for less than 3 per cent of world expenditure on biomedical research. Partly in response to the problem, the WHO has embarked on a new policy to speed up the delivery of new treatments to patients through collaboration with drug companies.

Tropical diseases	Countries affected	People infected (thousands)	People at risk (millions)
Malaria	103	270 000	2 100
Schistosomiasis	76	200 000	600
Lymphatic filariasis	76	90 000	900
River blindness	34	17 000	90
Chagas disease	21	16-18 000	90
Leishmaniasis	80	12 000	350
Leprosy	121	10-12 000	1 800
African sleeping sickness	36	25	50

The report is published by the WHO Special Programme for Research and Training in Tropical Diseases (TDR), which works with 5,000 researchers around the world to develop vaccines, drugs, diagnostic tests and methods for tropical diseases. The TDR is sponsored by the United Nations Development Programme and the World Bank.

Most of the people infected with tropical diseases live in countries with incomes of less than $400 per person each year. Their governments spend on average just $4 per person a year on health care. Pharmaceuticals companies are understandably reluctant to spend the $100 million needed to bring a drug or vaccine for a tropical disease to a market that cannot afford to pay for it. For the past decade, the TDR has been doing what drug companies cannot, funding basic research into tropical diseases. In that time, it has developed more than 100 potential products for treating or controlling tropical diseases.

It now has a vaccine to protect people against leishmaniasis under trial in Brazil and Iran. It is also testing the active component of an ancient Chinese herbal treatment for malaria, and traps in Uganda for tsetse flies, which carry the sleeping sickness parasite. The TDR has already developed biological insecticides for controlling the mosquitoes that carry filariasis (elephantiasis) in Indonesia and parts of Africa, and funded research that proved ivermectin, a drug originally developed for veterinary use, to be effective against the parasite that causes river blindness, or onchocerciasis.

But donors want to see the fruits of the TDR's research in the form of more drugs to treat people with the diseases. The TDR is responding by diverting money from basic research to drug development work that promises quicker results. (Source: New Scientist, 14 April 1990)

Regulatory issues

European Community agrees controls on biotechnology

Biotechnology companies will be subject to important new controls following the formal adoption of two directives in Brussels by EC environment ministers on 22 March. One of the directives deals with the contained use of genetically manipulated organisms (GMOs), while the other deals with the deliberate release of GMOs into the environment.

The directives lay down harmonized approval procedures to be followed ahead of experimental work, industrial production or the marketing of products. They are based closely on guidelines developed by the Organisation for Economic Co-operation and Development (OECD). (Source: Biotechnology Bulletin, Vol. 9, No. 3, April 1990)

General

ATCC news - culture catalogue for algae and protozoa

An update to the ATCC 1985 Catalogue of Protists is now available from the American Type Culture Collection (ATCC). The update, published in December 1989, contains descriptions of strains added to the collection since 1985. Two new features not found in the 1985 catalogue include:

(1) Complete media formulations to propagate all strains, including strains in the 1985 catalogue, and

(2) A set of special instructions providing detailed methods to culture and maintain certain strains.

The 23-page update also includes an index by ATCC number and a cross-reference index of strain designations used by other researchers. The cross-reference index is especially useful in locating strains cited in publications that are on deposit at the ATCC.

Culture catalogue update for fungi and yeasts

A new update to the ATCC 1987 Catalogue of Fungi and Yeasts is now available from the ATCC. The 63-page update contains descriptions of approximately 2,000 strains, representing over 860 species added to the collection since 1987. The update includes the following features:

(1) Strain descriptions arranged alphabetically by scientific name;

(2) ATCC recommended media formulations for revival and subculture;

(3) An index by ATCC number, and

(4) A cross-reference index of strain designations used by other collections or individuals.

The cross-reference index is especially useful in locating strains cited in publications that are on deposit at the ATCC.

Copies of both the above Catalogues are available free of charge from the ATCC, 12301 Parklawn Drive, Rockville, MD 20852, USA. You may also FAX a request to (301) 2312-5826. (Source: ABA Bulletin, Vol. 5, No. 2, April 1990)

Network set up for small firms

An international network, EuroBioMed has been formed to aid technology transfer and business co-operation between research institutes, companies and distributors in the field of biotechnology and medical technology.

The network is specifically aimed at linking small- and medium-sized European companies which have a commitment to international expansion and co-operation.

Organized by the RL Science Park Maastricht in the Netherlands, the network consists of a number of European companies, including BioResearch Ireland, and further members are being actively sought. It is supported by the EC Sprint programme. (Source: Manufacturing Chemist, April 1990)

Gene therapy: the struggle for acceptance

Scientists from the National Institutes of Health in the US last week began the long haul of wining permission for the first ever treatment of a disease with gene therapy. Ironically, in the same week, the Food and Drug Administration approved a conventional therapy for the same disease, and this could complicate efforts to secure backing for gene therapy.

The group, led by French Anderson and Michael Blaese, presented their experimental protocol to the Human Gene Therapy subcommittee of the NIH. Before the therapy can go ahead, this group and the Recombinant DNA Advisory Committee of the NIH must approve the protocol. So too must the Food and Drug Administration.

The researchers are seeking to treat a disease which affects babies, called severe combined immunodeficiency (SCID). Their immune systems cope poorly with a wide range of infections, such as pneumonia. About 25 per cent of those with SCID suffer from deficiency of an enzyme, adenosine deaminase (ADA), because of faults in the gene that manufactures it. ADA degrades certain biochemicals in the body. These products accumulate if ADA is absent. They interfere in some way with the synthesis of DNA, thus killing cells. Among the most prone are T cells, which are essential to the immune system.

Some patients are treated through bone marrow grafts, if suitable donors are available.

Alternatively, doctors can inject the enzyme directly into the body, but it soon degrades. The new conventional therapy approved last week is for a drug called PEG-ADA. This combines the enzyme with another molecule that enables the enzyme to survive intact for longer.

In its protocol, the gene therapy group points out that PEG-ADA does not constitute a cure for the disease. At best, the group says, it converts severe combined immunodeficiency to partial combined immunodeficiency. The gene therapy group plans to insert the gene that codes for ADA into a deactivated virus, incorporate the virus into T cells taken from the patient, then reinject the cells in a bid to cure the disease. (Source: New Scientist, 7 April 1990)

Genome scientists map out details of five-year plan

American biologists, geneticists and computer scientists have drawn up a detailed plan of how to develop the skills and techniques that will eventually allow them to sequence the entire human genome. The cost will be almost $1 billion over the next five years.

The plan includes the creation of up to 20 special centres, based primarily at universities and government research institutes, which will take on responsibility for particular aspects of the development work. It also covers the organisation of groups of scientists interested in studying the genetic characteristics of particular chromosomes, and a training programme that will eventually support up to 600 pre- and post-doctoral students.

If the proposed budget is approved by Congress, as much as $30 million of the total will be spent on studies of the social implications of this research, such as the way genetic information about an individual might be used or misused.

For the first five years, research will concentrate on scientific goals. These will include: improving the genetic and physical maps of all human chromosomes; mapping and beginning to sequence the DNA of model organisms such as bacteria, yeast, the nematode worm and mice; and reducing the cost of sequencing through technological innovations.

The five-year plan has been developed by the National Institutes of Health and the Department of Energy, the two federal agencies which first proposed the sequencing effort. The plan, which has been drawn up over the past two years, is currently in the final stages of preparation and is due to be published within the next few weeks.

The decision to provide substantial funding for research into the social implications of the project reflects the fact that this aspect has become increasingly controversial. The project raises a large number of ethical, legal and social issues. (Extracted from New Scientist, 24 February 1990)

Howard Hughes gets HUGO off the ground

Two years after it was founded, the Human Genome Organization (HUGO) has finally garnered its first funding of note. The Howard Hughes Medical Institute (HHMI) has announced a $1 million grant, spread over four years, to support HUGO's efforts to promote and co-ordinate international collaboration in mapping and sequencing the human genome.

A matching grant is expected to be announced soon by Burroughs Wellcome. With over $500,000 a year at its disposal, HUGO's first step will be to set up permanent offices (in Bethesda, Maryland, in London and in Osaka), and begin the work of helping to organize the 15-year $2,000-$3,000 million genome initative.

Without major funding, HUGO has so far been more concept than reality. But genome researchers hope that new grants will finally allow HUGO to take an active role in co-ordinating the exchange of data, samples and technology. (Source: Nature, Vol. 345, 10 May 1990)

Alternative agriculture

Over a year back, the National Research Council of the USA brought out a report titled "Alternate Agriculture" which caused a lot of interest and controversy. It was described by some as "The New Bible" of farming and "Resources of the Future" criticized it as an inaccurate and over-optimistic presentation. The synthetic fertilizer and pesticides industry was up in arms, for the altercation referred to the greater use of natural nutrients and pest control with reduced use of synthetics. The report was based as a study of the operation of a small section of farmers who developed and practised these alternate methods of farm production and gained significant sustained economic and environmental benefits. It was a "Systems approach" to farming that sought to develop multi-year practices that took advantage of all that could be produced on the farm and naturally occurring beneficial biological interactions. In effect, it meant expanded crop rotations and biological pest control, soil and water conservation and diversification in crop and livestock. Purchased inputs would be cut sharply and hence the outcry. The prime consideration were sustainable and ecological, regenerative farming operations.

There have been misgivings on the present methods of farming in the USA with a monocrop pattern based on heavy input of nutrients and pesticides. The choice was between high yields and price support or the alternative of keeping part of the land fallow for a subsidy paid by the US Government. Also there are environmental and potential health problems and possible pesticide residues on crops. Such farming is often felt as not sustainable over long periods. The earlier practices of crop rotation had given way to computerized input controls with high yields of the same crops. Ground water in some areas also gets contaminated with herbicides and residual run off nitrates which promote algal growth. The view is that erosion of soil is higher and even 20 times the rate of replenishment, or soil may get compacted and reduce root growth leading to lesser yields. As an extension of present farming practices, there is the use of antibiotics in animal feeds of about 10 million pounds a year now with repercussions on the health of the country. Agriculture is an industrial operation aimed at its one product and large operations fully mechanized, while shutting out the smaller farmers - receiving enormous amounts as subsidy. The results are no doubt impressive with corn using up 44 per cent and wheat 17 per cent of the annual nutrient consumption of 19.3 million tons in 1988-89.

What are the alternate farm practices? Ridge tillage system, crop rotation with a legume following a grain crop, deep-rooted crops such as alfalfa in rotation, synthetic fertilizer inputs as

supplements to composts on the basis of soil tests, integrated pest control with use of natural predator insects or pheromones, etc. Correct balance of nutrients is important. Apart from this, there is an integration with cattle farms and use of drugs. All these methods used by a select group of farmers are definitely beneficial on the long term in sustaining crop yields. although there is said to be no success with rice production. But the real problem in the USA is that the subsidy or price support schemes apply only to a few selected crops like feed grains, cotton, soyabeans and sugar. The main hurdle is to get enough flexibility in crop patterns and practices, so that the votaries of alternative farming methods are not penalized. (Extracted from Chemical Business, 5-19 April 1990)

Market for biocides up

Bullish predictions for the biocides market have been made by Rohm and Haas. Estimating the market at some $1 billion - $300 million in Europe - European marketing manager, Dr. Stuart Neal, says he expects "double digit growth into the 1990s" for the company's biocides division.

Neal bases his forecast on market potential, and adds "many market sectors and applications have yet to be penetrated, and customer needs for preservatives are always evolving in line with more sophisticated products".

One of the developments the company is particularly excited about is its new chemistry for wood and marine antifoulants paints, which was registered for use in Japan late last year, and is awaiting registration in Europe and the US. Few details of the product are available, but the company says it meets the need for a paint film preservative that will withstand tough climatic conditions without harming wildlife or the environment. (Source: Manufacturing Chemist, April 1990)

Fruits of the desert: a survival kit for the future

Economic botany is not a backwater where botanists dabble among weird and wonderful collections of arrowheads and carved beads. As its name suggests, it is about economics and is as relevant today as it ever was.

One area of activity that is growing increasingly urgent is to find plants that could improve the quality of life in some of the driest lands in the world. The arid and semi-arid zones are spreading, while the number of people living in them is increasing. And, as the world warms under the influence of the greenhouse effect, the marginal lands may grow still less hospitable.

One of the most important tasks for economic botanists is to identify the plants that local people use, however rarely, for one purpose or another. To this end, Kew has compiled a second database for economic botany; an archive that holds details of 5,000 species that people use for food, forage or fuel in arid and semi-arid lands. Botanists built up a record of these plants for the Survey of Economic Plants for Arid and Semi-Arid Lands project, begun after the disastrous droughts that plagued Africa in the late 1970s.

The next phase of the project involves collecting seeds of these plants from their natural habitats, growing them in the laboratory and learning more about them. Seed collectors based at Wakehurst Place, the Royal Botanical gardens at Kew's outpost in Sussex, have begun to concentrate their efforts on species that seem to have the most potential.

A wide range of plants could help the people living in marginal lands. Many fruits and vegetables are eaten only occasionally. Some could be valuable as a more regular part of the diet. One such species is Cordeauxia edulis, from the border of Ethiopia and Somalia. This plant produces the ye'eb nut, which tastes rather like a sweet peanut. Local people eat the nuts when they come across them and their animals browse on the shrubby plant. According to David Field, head of the economic and conservation section at Kew, the ye'eb could become the next macademia nut, providing an income for local people as well as food.

Another plant has already shown its potential. The root of the grapple plant, Harpagophytum procumbens, is a remedy for just about every disorder. Already, there is a large market for the root in Europe. Thousands of tonnes of grapple roots have been exported from the Kalahari in Namibia and the plant is seriously over-exploited. Without a programme to replant the grapple, it could soon disappear from the local array of remedies.

Before beginning programmes to grow plants known to be useful, scientists need to learn more about how to cultivate them. A newly discovered species of locust bean from Oman would be an ideal plant for arid lands. It is drought resistant, more productive than the Mediterranean carob and makes good forage. Unfortunately it is very rare and it is difficult to grow. Researchers have only been able to raise this species using micropropagation. "This species is vulnerable in its natural habitat, but potentially it is a very useful plant for the tropics", says Field. If its potential is not realized soon it could disappear.

Choosing plants for arid lands is not simply a question of looking at what people eat in dire straits. Some foods that people turn to in times of famine are a last resort. They often contain toxins, which people can tolerate for a short time. With more research however, it might be possible to select strains that do not contain toxins and make a palatable everyday food.

In many of the dry lands, growing food plants is not the biggest priority. The first step is to stabilize and improve the soils of the desert. Frances Crook, a colleague of Field's, is looking for plants that form a "green glue", holding back the desert and increasing the fertility of the soil. The legumes Ast-agalus kentophyta and Tephrosia vogelii, which fix nitrogen, are especially good at doing both. The researchers at Kew are focusing their attentions on plants that have more than one use, providing fuel and an edible fruit or gum for example. The more benefits a plant brings the more likely local people are to plant them.

Kew hopes that aid agencies, local organizations and village projects will take the information it provides and set up planting trials. It would also like to maintain a close involvement. "Kew would like to send teams that include ecologists, anthronologists and ethnobotanists to bring together all the information on the plants and the people who use them", said Field. "This will help in designing orogrammes for planting." In the

early days of the arid and semi-arid lands project, the researchers received funding from Oxfam and the Clothworkers Foundation. Now that it has reached a practical stage of its work, it desperately needs more support. (Source: New Scientist, 17 March 1990)

B. COUNTRY NEWS

Australia

Proposal to establish genomic information service

Genome mapping in human, animal and plant spheres has been targeted for a concerted effort over the next fifteen years, particularly in the United States. A major focus for the US (and international) effort is the Human Genomes (HUGO) project. Proposals are being examined by the Australian Government through the International Division of the Department of Industry, Technology & Commerce for a National Genomic Information Service (NGIS). It is proposed that this be established to specifically support genome studies and biomedical research. The concept is that NGIS will acquire and maintain Australian and overseas data bases and computer facilities necessary for the operation of the data bases. It will provide access to the data bases for all Australian scientists and access to computational expertise to assist in the interpretation of the analysis. NGIS will be accessible through AUSTPAC, AARNET and direct connection.

Computing facilities and at least some expertise will be located within an existing institution which would be expected to contribute to the capital and/or operating costs in return for its use of the facility. The Walter and Eliza Hall Institute and CSIRO Division of Biomolecular Engineering (Sydney) have offered their services. Other sources of expertise may be distributed at up to three "nodes" within the NGIS. The Walter and Eliza Hall Institute, CSIRO Division of Biomolecular Engineering (Sydney) and The Research School of Biological Sciences (ANU) are possible sites.

Capital costs of NGIS will be sought from appropriate federal funding bodies. Operating costs will also be sought on a diminishing scale for three years. Access fees will be charged. A flat fee (which could be as high as $A10,000 per user account depending on services offered) is simple to administer and would cover a substantial portion of operating expenses. A premium would be levied on commercial users. (Source: ABA Bulletin, Vol. 5, No. 2, April 1990)

New GMAC guidelines

GMAC has now published the "Guidelines for Small Scale Genetic Manipulation Work" which have been under discussion for some time. These apply to work involving culture volumes of less than 10 litres. Depending on the nature of the work and the perceived degree of risk, the work may be categorized as A, B or C, and different approval procedures are detailed for each. The document also deals with the role and responsibility of GMAC, Institutional Biosafety Committees and Principal Investigators as well as importation and transportation of samples. The appendices cover other relevant documents including other related Australian guidelines and regulations, a list of organisms known to exchange DNA by known physiological processes, a list of GMAC approved host/vector systems, instructions for completing GMAC proposal forms, a list of some potent toxins, requirements for work with live viral vectors, procedures for work with hazardous fragments of DNA, extra information required for whole plant work, requirements for physical containment levels C1, C2 and C3, requirements for plant house levels PH1, PH2 and PH3, requirements for experiments involving transgenic animals and animal containment facilities levels C1 and C2, contact details for various transport authorities and a list of exotic organisms whose entry to Australia is forbidden.

Copies of these Guidelines and the appropriate proposal and assessment forms may be obtained from: GMAC Secretariat, P.O. Box 2183, Canberra, ACT 2601 (Tel: (062) 57 3441). (Source: ABA Bulletin, Vol. 5, No. 2, April 1990)

Microbial safety

A new "draft Australian Standard for Comment" has been released by Standards Australia on Safety in Laboratories. Part 3: Microbiology (Revision of AS2243.3 - 1985). This document provides a good guide to safe working practices in microbiology. All biotechnology organizations using microbiology should be aware of this publication. (Source: ABA Bulletin, Vol. 5, No. 2, April 1990)

Genetically altered meat slips through the net

Australia's leading environmental pressure group has called for legislation following accusations that 53 genetically altered pigs were transported to market and sold for human consumption without proper approval. The pigs were not dangerous to human health, but scientists at the University of Adelaide and their commercial partners, Metrotec, have been accused of transporting the pigs without permission.

Both groups deny the charge and say that the Australian Conservation Foundation has blown the case out of proportion. The ACF says that moving the pigs to market was equivalent to an unauthorized release of a genetically engineered organism.

"The ACF has the objective of closing down all recombinant DNA research in Australia," said Bob Seamark, an endocrinologist from the University of Adelaide. The ACF is believed to be using the case to push the establishment in Australia of a national environmental protection agency, along the lines of the American Environmental Protection Agency.

The case has caused a furore both in Australia, and worldwide. One of the main issues is whether scientists should have their work controlled by legislation or be expected to abide by the sort of voluntary guidelines that exist in Australia.

Earlier in May, the Australian Government's Genetic Manipulation Advisory Committee was due to debate the need for legislation. The secretary of the GMAC, Vimala Sarma, said that guidelines were preferable to legislation because they could be more readily altered in response to the ever-changing state of research into recombinant DNA.

The committee will also consider the possibility of recommending sanctions against the university scientists and Metrotec. Government funding for the research could be withdrawn on the recommendation of the GMAC. The project has an A$800,000 (£400,000) government grant, according to the ACF.

Seamark is regarded as a world leader in the development of transgenic animals. Since 1982, he has been using genetic engineering techniques to breed leaner pigs that require less feed than normal pigs and grow more quickly. The "super pig" he is developing is designed to achieve market weight in 17 weeks instead of 25 weeks, on a third less food.

The pig project involves adding to embryos taken from a sow, extra copies of the gene responsible for the production of growth hormone in pigs. The embryos are returned for normal gestation. Some of the offspring grow faster because they express extra amounts of growth hormone.

In April 1988, Metrotec transported 53 pigs to the Mount Barker abattoir. They were the offspring of a boar that had been genetically altered. The boar and these offspring carried the gene but did not express it. The pigs were no longer required for the project. They were regarded as safe to consume because they did not express the hormone from the additional gene. (Source: New Scientist, 12 May 1990)

European Community

New European release rules ratified

European Community environment ministers have approved two directives controlling the use of genetically manipulated organisms (GMOs). The directives, opposed by leading European biotechnology companies set out strict rules for the use of GMOs in contained situations, and for environmental releases. Member States have 18 months to implement the directives through national legislation.

All installations using GMOs must be registered, but subsequent authorizations to work with particular GMOs will depend on whether the installation is a research laboratory or an industrial plant, as well as on an assessment of the risk the organisms pose to health. Industrial plants will face tougher controls, and must obtain explicit authorizations to work with "high risk" (including pathogenic) GMOs.

Companies or laboratories proposing releases of GMOs must provide an assessment of the environmental hazards. Experimental releases will have to be cleared only in the country concerned, but the release of live GMOs in commercial products must be referred to the European Commission and other member States.

If some countries object to specific products, an arbitration procedure will come into play, but authorization, once given, will apply to all Community member States. (Source: Nature, Vol. 344, 29 March 1990)

European firms support sequencing yeast genome

Sixteen European companies that use yeast in their business have agreed to organize a loose consortium to profit by mapping and sequencing the genome of baker's yeast - Saccharomyces cerevisiae. European Community representatives projected a "Yeast Industry Platform" to encourage the EEC's newly launched four-year, $23.5 million programme to map and sequence the 16-chromosome yeast genome.

"A realistic scenario for the Platform," says Alessio Vassarotti, manager of the project at EC's Directorate of Science, R&D, Biotechnology Division, "could consist of interested companies contributing an annual fee, in return for privileged access to the sequencing data produced by the 35 laboratory network - that is, during the last six months in which data are not yet in the public domain."

The European Community is financing the yeast-genome-mapping endeavour, mainly through a programme called BRIDGE - Biotechnology Research Innovation Growth in Europe. It has earmarked seven million ECUs (European currency units), equivalent to $8.5 million, to fund the mapping and sequencing of three or four new yeast chromosomes by 1995. (Extracted from McGraw-Hill's Biotechnology Newswatch, 19 March 1990)

European biotechnology programmes and developing countries

Biotechnology stimulation programmes of the European Community aim at making Europe internationally more competitive. The interests of developing countries, whose trade flows may change as a consequence of these programmes, are often not sufficiently taken into account. The parallel programme of "Science and Technology for Development" covers the same fields of technology, but is hardly linked to the biotechnology stimulation programmes BRIDGE, FLAIR and ECLAIR.

BEP and BAP

Since the beginning of the 1980s the European Community (EC) has promoted biotechnological research and training. Starting with the Biomolecular Engineering Programme (BEP) in 1982 the EC has launched several biotechnology stimulation programmes. The main objective of BEP was to contribute to the removal of bottlenecks that inhibit application of modern biochemistry and molecular genetics in certain sectors of the agro-food industry and agriculture.

In 1985 the Biotechnology Action Programme (BAP) was implemented. This programme was both a continuation of BEP and an extension into new areas considered as essential for the development of biotechnology in the Community. The new domains include enzyme engineering, application of genetic engineering to industrial micro-organisms, in vitro testing systems, bio-informatics, etc.

The goal of the EC is to improve the competitiveness of European industry and agriculture. Although the financial volume of both programmes is small in comparison with national efforts in the various member States of the EC, they play an important role. Their emphasis is on transnational co-operation in biotechnology R&D. They are complementary to national activities, and can improve their effectiveness through better co-ordination and a reduction of overlap.

BRIDGE

A follow-up to the BAP is the new research and development programme named BRIDGE (Biotechnology Research for Innovation, Development and Growth in Europe). This programme, which runs from 1990 until 1993, was approved by the Council of Ministers on 9 December 1989.

BRIDGE is subdivided, as was BAP, into two actions: Action I is for research and training, emphasizing transnational collaborative research. This section is subdivided in four sectors: information infrastructures, enabling technologies (e.g. protein engineering), cellular biology and pre-normative research (e.g. safety assessment).

Action II is for accompanying measures of "Concertation": co-ordination of Commission activities, concertation of member States' activities, providing information, monitoring and interpreting world-wide developments, etc.

FLAIR and ECLAIR

BRIDGE is very closely related to two more downstream EC technology programmes, ECLAIR and FLAIR. ECLAIR (European Collaborative Linkage of Agriculture and Industry through Research) is aimed at improving the interface between agriculture and industry by way of pre-competitive projects based on recent advances in life sciences and biotechnology. This programme, approved by the Council of the EC in February, covers three broad themes:

- Improvement of existing agricultural products and development of new products for industrial use in order to find new markets and applications;
- Development of more selective and efficient agricultural inputs, and more selective and efficient industrial processing of agricultural products;
- Development of integrated agro-industrial projects, like total crop harvesting.

Quite opposite to traditional agro-industrial relations where industry processes what agriculture produces, demands from industry are the starting point in the ECLAIR programme.

FLAIR (Food-Linked Agro-Industrial Research) is more than a biotechnology programme, it is dealing with all food sciences and technology. Support will be given to research on food quality, nutritional and health aspects, and safety and toxicological aspects of food. FLAIR was approved by the Council on 20 June 1989.

Developing countries

The European Community is the largest importer of products from developing countries, with agricultural products making up a large part of this trade. Therefore, it is important for these countries to know what impact biotechnology applications will have on their exports to the EC. The general aim of the EC biotechnology programmes to enhance the EC's competitiveness may lead to higher rates of self-sufficiency. This in turn may lead to less imports from developing countries. For instance, in the ECLAIR programme research is proposed to make soya better suitable for the European climate. Thus, the outcome of these programmes will have its effect on the trade of developing countries (in particular from Brazil and Argentina).

The interests of developing countries generally are not taken into account in the biotechnology programmes. Only in the Concertation part of BAP some initiatives of interest for developing countries were launched, like the Seminar on Biotechnology in Europe and Latin America for industrial collaboration. Although in the proposal for the BRIDGE programme the relationship between this programme and other R&D Community activities like the programme Science and Technology for Development is mentioned, there seems to be little co-ordination at project level.

This lack of attention can be explained by the differential phasing of programmes, e.g. ECLAIR and FLAIR started in 1989, BRIDGE and the new AGRICULTURE programme have not yet started, while STD 2 was approved at the end of 1987. Thus, STD funds were largely used up before the other programmes were properly in progress. Co-ordination should be improved in the future.

The Commission has stressed at different times that developments in European agriculture and industry should not jeopardize developing countries' interests. In a June 1986 Commission document it was said that "if Europe were to reduce significantly its dependence upon imports of agricultural raw materials, this would undoubtedly have serious impacts on agriculture and the economies (and, hence, possible land use and environmental developments) in developing countries. It is therefore necessary (in parallel with our own evolution towards the new agro-industrial phase of our own development) to continue and reinforce existing Community efforts to assist these countries to improve their agriculture and to secure environmentally sustainable rural development. Biotechnology may well be able to contribute substantially to these aims."

Science and technology for development

When talking about developing countries' interests in relation to EC technology stimulation, EC officials refer to the Science and Technology for Development (STD) programme. This programme has been set up in order to support scientific activities for the benefit of developing countries, by means of joint research contracts.

The first STD programme was launched in 1983 and covered the period 1983-1986. The programme concerned two areas of work of vital importance for developing countries: agriculture and health. This first STD programme was allocated 40 million ECU and financed 411 research projects.

For the period 1987-1990 a second STD was approved in December 1987. This programme covers the same areas as its predecessor: "tropical and subtropical agriculture", and "medicine health and nutrition in tropical and subtropical areas". This second STD programme, which was allocated 80 million ECU, has the explicit objective of strengthening scientific capacities in third world countries through the forging of closer links between laboratories in the North and the South.

Biotechnology, of course, is a major technology in both subprogrammes, tropical agriculture and health and nutrition. However, biotechnology is not explicitly mentioned in the programme guidelines for STD-2, which is dealing with a broad range of technologies important to developing countries.

One may expect close interaction between STD-2 and programmes like ECLAIR and FLAIR since the latter two are dealing with very similar technologies and fields of application (agriculture and food processing). Particularly in the field of agricultural biotechnology research options for integration of these programmes do exist. However, such integration does not occur, although all these programmes were conceived and are managed by the same Directorate-General of the Commission (DG XII: Science, Research and Development).

Integration of science and technology programmes for Europe and those for developing countries is even more necessary as the development gap between North and South will probably increase in the next 15-20 years. Intensifying the use of

science and technology in the economies of the industrialized countries may lead to more unequal exchange between the North and the South. Moreover, the growing importance of biological diversity as a prerequisite for agro-industrial development is asking for not just a European but a global action in scientific, technological and industrial development.

Science and technology for development

Outline of Programme

A. Subprogramme: Tropical and subtropical agriculture

1. Improvement of agricultural products:

   - Crop production: food and agro-industrial crops; crop genetics; crop protection;

   - Livestock production and fisheries: stock-farming; animal genetics and reproduction; veterinary medicine; sea and inland fisheries, aquaculture;

   - Improved forestry production in both humid and arid areas.

2. Conservation and better use of the environment:

   - Appraisal of resources: water resources and their use, soil management and protection; better management of fragile environments.

3. Agricultural engineering and post-harvest technology:

   - Agricultural engineering/mechanization; product conservation and processing.

4. Production systems:

   - Crop systems; production systems.

B. Subprogramme: Medicine, health and nutrition in (sub)tropical areas

1. Medicine:

   - Infectious tropical diseases: parasitological, bacterial, viral and mycological diseases;

   - Non-infectious tropical dieseases; genetic disorders; acquired diseases.

2. Health:

   - Health services: operational research, organization, management and models;

   - Environmental health: water-related diseases;

   - Traditional medicine: medicinal plants.

3. Nutrition:

   - Nutritional deficiencies;

   - Impact of agricultural, food and socio-economic strategies on nutrition;

   - Relationships between production, storage systems, food habits and state of health of the population;

   - Bio-availability of nutrients.

New framework programme

In the new framework programme of Community activities in the field of research and technological development (1990-1994), 714 million ECU will be devoted to the Life Sciences and Technologies programme. Within the latter 111 million ECU will be spent on research and development in life sciences and technologies for developing countries. Emphasis is placed on tropical agriculture (integrated management of agricultural resources for reducing food shortages in regions at risk whilst protecting the environment) and on tropical health research (efforts are concentrated on new steps to combat some major tropical diseases).

The Commission has stressed its dedication towards integration of the constituent programmes within the total framework programme. So far, only the financial aspects of the new framework programme have been approved: 5.7 million ECU for the total programme (Council decision of 15 December 1989). Whether the projects for developing countries will be more integrated with those for biotechnology and those for agriculture is still to be seen.

For more information on the biotechnology programmes of the European Community contact: Dr. Mark Cantley, Concertation Unit for Biotechnology in Europe, Commission of the European Communities, Rue de la Loi 200, B-1049, Brussels, Belgium. (Source: Biotechnology and Development Monitor, No. 2, March 1990)

Federal Republic of Germany

FRG close to passing gene law

The FRG's planned legislation covering work with genetically manipulated micro-organisms has taken its penultimate step.

The bill, redrafted by the health ministry after its rejection by the upper house of parliament, the Bundesrat, last summer, has now passed the lower house, the Bundestag. It is scheduled to be debated again on 11 May by the Bundesrat, which had demanded more than 250 changes to the original draft. If passed by the Bundesrat the law could take effect on 1 July.

While the German legislation will regulate industrial production and research work with genetic technologies, it will not apply to questions of genome analysis or genetic therapy. The release of manipulated micro-organisms into the environment will continue to be subject to approval by the federal health authority.

The federal states will continue to have responsibility for approving new plants working with genetic technologies. The bill stipulates that a decision must be made within three months. (Source: European Chemical News, 9 April 1990)

Schering plans to boost biotechnology by Codon takeover

Schering has announced plans to acquire US biotechnology firm Codon, based in south San Francisco, for an undisclosed sum.

The acquisition, which has been approved by all of Codon's administrative units and by the majority of its shareholders, is expected to be completed within the next few weeks.

For Schering, the takeover of Codon, with which it has co-operated in the past, will represent a first step towards what is planned to be a "considerable expansion" of its biotechnology activities, the company said. According to a spokesman, the acquisition of Codon will help the West Berlin-based pharmaceuticals and agro-chemicals major to gain a foothold in the important US genetic engineering sector and will allow it to shape the business according to its needs.

Codon, which employs 100 people and specializes in the development and production of proteins for human and veterinary drugs, holds patents for an anti-coagulant protein, in which Schering is particularly interested. (Source: European Chemical News, 14 May 1990)

France

New company to exploit technique

France's Centre Nationale de Transfusion Sanguine has set up an affiliate company to exploit a technique of introducing therapeutic chemicals into red blood cells. Novacell, located in Tours, aims to develop the technology which, by the introduction of drugs or enzymes, could be used to improve the oxygenation of tissues, fight leukaemia and boost the detoxification of alcohol in the blood. It is developing two projects: one increasing the efficiency of the blood's oxygen potential, and another using cells as vectors for immunomodulators, suppressants and vaccines. (Source: European Chemical News, 2 April 1990)

Japan

Government biotechnology funding

Government funding for biotechnology R&D totalled about $600 million in 1989. The government biotechnology policy has emphasized government/industry collaboration through research associations, independent research groups and projects involving government, industry and university researchers. Government/industry biotechnology R&D programmes received $27.8 million in funds in 1989. Agriculture and food processing R&D received $22.3 million; energy and bulk chemical R&D, $10.3 million; pharmaceutical R&D, $8.2 million; waste treatment R&D, $17.4 million; the ERATO programme, $10.4 million; and the Protein Engineering Center programme, $8.2 million. About 80 per cent of Japanese biotechnology R&D is funded by the private sector. Japanese biotechnology firms are moving towards international activities, primarily through joint ventures and acquisitions in foreign markets. The activities open foreign markets to Japanese products, and they give Japanese fims access to products developed by foreign companies. Currently, Japanese pharmaceutical export sales account for only 6 per cent of total sales. The top 11 Japanese pharmaceutical companies spend about 8.7 per cent of sales on R&D, a much lower percentage than Western pharmaceutical firms. (Source: Technology Update, 9 April 1990)

Rice genome

Japan is planning a long-term international project to map the rice plant's genome. If approved, the project will begin in 1991 and extend into the next century, involving researchers throughout Asia.

During its first 10 years, the project will aim to identify 1,000 chromosomal marker genes and develop methods to introduce new genes into specific places. Researchers will then turn to sequencing the more than 100 million base pairs which make up the genome.

The Rice Genome Council, drawn from universities and national research institutes, met to plan the scheme. The project is likely to win support: rice farmers make up the most powerful political constituency in Japan. Japan's state-owned Japan Tobacco (JT) and Belgium-based Plant Genetic Systems (PGS) are to collaborate on the development and commercialization of genetically engineered hybrids of rice. Research will be carried out at a PGS and JT's plant improvement and genetic research centre. Since April 1989 JT has held a 12 per cent stake in PGS. (Source: New Scientist, 10 February 1990 and European Chemical News, 7 May 1990)

Norway

Norway backs bioprotein project

A Norwegian consortium, which includes pharmaceuticals group Hafslund Nycomed, Statoil and venture capital company Norsk Vekst, is behind a new project to invest in the production of a bioprotein from natural gas. The process is being developed by Danish firm Dansk Bioprotein.

The bioprotein under development has a single cell structure and a protein content equivalent to that of fishmeal. Dansk Bioprotein has developed the production technique, which uses bacterial cultures to convert methane gas into biomass with 70 per cent protein.

The consortium has described future market prospects as "highly promising". It is expected that the product will be aimed primarily at the fish feed, sea farm and aquaculture industries, providing a new outlet for use of Statoil's substantial natural gas reserves.

The consortium plans to acquire a 56 per cent holding in Dansk Bioprotein with an option to raise joint ownership to 70 per cent within a two-year period.

The initial investment is Dkr 50 million.

The consortium is considering an investment in a bioprotein production facility at Statoil's west coast natural gas processing terminal at Karsto. Any decision to invest will be tied to economic factors and conditional on the successful development of the bioprotein process for commercial utilization. (Source: European Chemical News, 7 May 1990)

Sweden

Special permit needed for cultivation of gene manipulated plants

The growing of plants changed with the aid of gene technology will in the future require a special permit, according to a decision by the Swedish Government. Permits will be issued by the National Board of Agriculture after consultation with the Delegation for Recombinant-DNA and the National Environment Protection Board.

The Board of Agriculture is to make a risk assessment before any permit can be issued. Should the cultivation involve risks to the environment a permit is not granted, as will be the case if the genetic variety of nature is threatened, it is stated. (Source: SIP, March 1990)

Bioscientific research centre opened near Stockholm

A new research centre for biosciences was officially inaugurated by King Carl Gustaf at Huddinge, a Stockholm suburb, in February. Called Novum and located adjacent to Huddinge University Hospital and the southern campus of the Karolinska Institute, which includes a dental college, the centre is said to be the largest research and development venture in the fields of biotechnology and medical and dental technology in northern Europe.

Among the Novum partners are: the Centre for Biotechnology (CBT), created five years ago by the Karolinska Institute, the Stockholm County Council and the business community and already one of Europe's major research centres in its field; the Centre for Dental Technology and Biomaterials (CDB), specializing in diagnostic and therapeutic methods in dentistry and health care; and the Centre for Nutrition and Toxicology (CNT), intended to create a link with molecular biology research.

The commercial partners include Karo Bio AB, a company developing pharmaceuticals and diagnostics, notably steroid hormones, drugs against osteoporosis, and new systems for protein administration, and Kabi Invent AB, a development company in the Kabi Group creating new products allowing safer and simpler use of drugs.

Novum also houses two medical research institutes, one for medical nutrition and one for medical technology. The former is cross-disciplinary and covers such specialities as endocrinology, biochemistry, toxicology and cancer research. NutriSystem AB is currently building the first nutritional laboratory of its kind, where the nutritional content of foodstuffs will be analysed using computer technology. Its kitchen will then be able to change components while retaining the culinary qualities.

Novum research centre will be part of a large research village and some of its institutions will be included in a planned university for the Södertörn region. The next centre now taking form is called Bruket and has its architectural roots in the tradition of the Swedish "bruk", a small rural community grouped around an iron- or wood-processing mill and its manor. It will encompass some 14,000 sq. m of offices, laboratories and workshops and will open in 1991. (Source: SIP, March 1990)

Switzerland

Ciba seeks biotech approval

Ciba-Geigy (Basel) has applied for permission from regional authorities to build an $80 million biotechnology research and development centre in Basel. The Biotechnikum will employ about 100 people when it comes on stream at the end of 1992, and house research laboratories and a development plant to make a number of substances using fermentation techniques. These will include antibiotics, interferons for viral infections in humans and animals, hirudin and fibrinolytics for prevention and dissolution of blood clots, and elastase inhibitors to fight lung disease. A response from cantonal authorities is expected to take several months as protesters have filed hundreds of formal objections to the biotechnology centre. (Source: Chemical Week, 7 March 1990)

United Kingdom

UK Genetics Forum opposes bio-information restrictions

The public were not told about a plan to make bread with genetically engineered yeast until after the go-ahead had been given for the commercial use of the yeast, says the UK Genetics Forum.

On the same day (1 March 1990), the Government confirmed its refusal to write provisions for public access to information on genetically modified organisms (GMOs) into the Environmental Protection Bill, dubbed the "Green Bill".

Although the Government has announced its intention to set up public information registers, the quality and quantity of information will be entirely at the discretion of the relevant Secretary of State.

The UK Genetic Forum has repeated its call to the Government to set up a Genetic Modification Commission, in addition to the committee that will grant approvals for genetic modification experiments and products. The Commission, the Forum argues, should be a public forum for the discussion of the far-reaching implications of genetic technologies. It should include representatives of a broad range of interest groups, including environmentalists, consumers, the churches, health specialists and the biotechnology industry. Details from: Nick Rowcliffe, UK Genetics Forum, on 01-278 7624. (Source: Biotechnology Bulletin, Vol. 9, No. 3, April 1990)

Ministers maintain secrecy over genetic releases

The British Government is refusing to back down in the face of demands for access to information on genetically modified organisms, despite major concessions on public information concerning most other aspects of pollution. This anomaly has emerged as the Department of the Environment's "Green Bill" reached a critical stage in parliament this week.

MPs are in the process of debating a series of amendments to the Environmental Protection Bill. Amendments already accepted by the Government represent a major breakthrough in the amount of information made public on almost all industrial pollution. Those still under debate will, if agreed as expected, mean the Government has now conceded that local authorities should hold registers of all contaminated land in their administrative areas.

The pollution registers will be kept by regulatory authorities such as Her Majesty's Inspectorate of Pollution, the National Rivers Authority, and local councils. But the products of genetic engineering have been left out of this process.

Instead, the Government has handed to ministers the right to decide how much information on the release of these organisms to make public. The Department of the Environment says it would "proceed

from a presumption in favour of releasing information", but may decide "judiciously to limit the information as circumstances dictate". (Source: New Scientist, 5 May 1990)

United States of America

Leading biotechnology companies quit the IBA

The resignation of Genetics Institute and Cetus Corporation from the Industrial Biotechnology Association (IBA), following shortly after the withdrawal of another member, the Upjohn Company, is seen as a major loss to the association. Both companies to leave were founding members of the IBA in 1981 and are prominent in the industry. In a letter to Richard Godown, president of the IBA, Robert Fildes, president and chief executive officer of Cetus, stated that "the IBA has too frequently become the mouthpiece for a few companies whose positions do not represent those of all the members". The final straw seems to have been a vote taken by board members on 21 February to oppose changes in the 1983 Orphan Drug Act, as well as to support a bill now before a House of Representatives subcommittee, which proposes to strengthen US patent protection for genetically engineered products against unfair foreign competition.

The Orphan Drug Act was designed to provide tax incentives and a seven-year marketing monopoly in the United States to companies developing drugs for rare diseases affecting fewer than 200,000 patients.

Also at issue is the "Boucher bill", introduced by Representative Rich Boucher (Democrat, Virginia). The bill would provide the International Trade Commission with the authority to exclude foreign products made using a host cell, DNA sequence, or vector patented in the United States, closing what the bill's sponsors believe to be an unfair legal loophole.

Genetics Institute would be affected by the legislation, as it would prevent the importation into the United States of its EPO product, which is produced in Japan by its licensee, Chugai Pharmaceuticals. (Source: Nature, Vol. 344, 5 April 1990)

Transfer study expands

A subcomittee of the US Recombinant DNA Advisory Committee (RAC) lifted restrictions on the number of patients for a key gene transfer trial, but postponed a decision on an application for the first gene therapy experiment.

At the meeting of a subcommittee of RAC, members voted unanimously to lift the cap on patient numbers, now set at 10, for the continuing gene transfer study of patients with widespread melanoma. Although, in principle, the patient-number limitation has been removed, the Institutional Biosafety Committee (IBC) of the National Institutes of Health (NIH) has requested a temporary cap of 50 patients.

The study, sanctioned in January 1989 after a lengthy federal review process, is a joint effort between W. French Anderson of the Heart, Lung and Blood Institute and R. Michael Blaese and Steven A. Rosenberg of the National Cancer Institute (NCI). It involves readministering interleukin-2-stimulated T-lymphocytes marked with the gene for neomycin resistance to patients from whom the cells were isolated. Although the gene confers no therapeutic benefit, it allows the researchers to track the cancer-killing tumour-infiltrating lymphocytes (TIL) in the patient's body. So far, six of the 10 patients have been treated, with data on the first five submitted to the New England Journal of Medicine. The TIL appear to "home" to the tumour site, but whether this co-relates with anti-tumour activity has yet to be determined.

Now that researchers have established that the gene transfer protocol is safe, they hope to introduce genes of therapeutic value, such as tumour necrosis factor and interferon. Rosenberg will be submitting the next phase of the TIL protocol for review within two months, but because the subcommittee and the RAC meet infrequently the protocol will be subject to delays of six months or more. (Extracted from Nature, Vol. 344, 5 April 1990)

Pilot plant for University

Pennsylvania State University has opened a bioprocessing pilot plant at its University Park site, with a view to offering expertise and equipment to move biotechnology into mass production, a university spokesman says. The pilot plant's first customer is Bio-Protecus, a Reading, Pa. company that is looking at biological methods of waste disposal.

The rationale for the unit is that small- to medium-sized companies developing new products cannot afford the capital investment necessary for their own pilot plants. (Source: Chemical Marketing Reporter, 21 May 1990)

Health worries over use of milk hormone

Complaints by small dairy farms that a genetically engineered hormone designed to boost milk production could put them out of business has led two US states temporarily to ban the product.

The governors of Minnesota and Wisconsin signed legislation that places a ban until mid-1991 on the sale or use of bovine somatotropin (BST) in the two states, which, combined, produce 20 per cent of the United States' milk. Wisconsin Governor Tommy Thompson said that the ban would allow "additional farmer and consumer education" without hurting the state economically. Vermont's legislature last week rejected a similar proposal.

Opponents of the hormone claim that it encourages overproduction, which would flood the market and eventually drive out small dairy farmers.

Health worries remain an issue in the debate. Although the US Food and Drug Administration (FDA) says that its studies show that the hormone presents no danger to the public, it has not yet published the data behind that claim. Beyond any possible impact on humans, BST appears to shorten the life of the cow. Although milk production can rise 10 to 25 per cent "cow burnout" can cut reproductive life-spans by a third, he says.

Four biotechnology companies - Monsanto, Eli Lilly, American Cyanamid and Upjohn - have spent more than $500 million in developing the drug. But BST has been plagued by public opposition and controversy since field trials began in 1985. Last year 2,500 US supermarkets agreed to boycott BST-produced milk. Until FDA approves BST for

general sale and use, which the agency plans to do early next year, the supermarkets say they will not sell the milk. (Source: Nature, Vol. 345, 24 May 1990)

Japanese/US joint venture announced

Consolidation within the biotechnology industry continues apace with the announcement this week that US-based Genetics Institute is entering into a joint venture with Yamanouchi Pharmaceutical Company Ltd., one of Japan's largest drug companies. The two-part agreement involves the formation of a separate company for the commercialization and marketing of Genetics Institute's genetically engineered bone morphogenetic proteins (BMPs) in Japan, and the setting up of a partnership to support the development and commercialization of BMP's worldwide.

As a result, Genetics Institute is likely to become the first US biotechnology company to establish a direct marketing presence in Japan. In the agreements, Genetics Institute will retain worldwide manufacturing rights and US marketing rights to its BMP products, and will receive a 50 per cent share in the profits from the sale of its BMPs in Japan. (Source: Nature, Vol. 345, 24 May 1990)

C. RESEARCH

Research on human genes

Ethical dilemmas avoided by tests on unfertilized eggs

A new way of preventing the birth of children with severe genetic diseases, while avoiding some of the ethical difficulties associated with looking for genetic defects in fertilized human embryos, has been developed by researchers in London.

The technique, developed by Marilyn Monk and Cathy Holding of the Medical Research Council's Mammalian Development Unit in London, would enable researchers to test the unfertilized eggs of a woman known to be the carrier of a genetic defect.

Only those eggs found by such a test to be free of the disease-causing gene would be fertilized. As a result, this approach may obviate the need for diagnostic procedures on embryos.

A separate group of research workers at the Hammersmith Hospital in London recently announced that they had successfully tested eight-cell human embryos for specific genetic diseases. They removed one cell for tests, without damaging the rest of the embryo.

Analysing the genes of an unfertilized egg is more difficult, since it is a single cell - and the removal of its DNA would kill it. Monk and Holding have exploited the fact that there is a source of expendable DNA in a cellular element called the first polar body. When an egg cell matures, the chromosomes it contains double and then separate; one set of chromosomes is then ejected from the egg as the first polar body.

If a woman is the carrier of a defective gene, then the mutation will normally occur in the chromosomes either in the polar body, or in the egg. In other words, the polar body contains genetic information not only about itself, but also about the egg.

The presence of the mutation in the polar body will indicate that the egg is unlikely to be carrying the defect, say the researchers. "Conversely, if the polar body is normal, the egg is probably defective and should not be fertilized."

The only complication is that a chromosomal rearrangement called "crossing over" may have happened. In this case, the polar body will have one copy of the defective gene instead of two. This would indicate that the egg has a 50 per cent chance of transmitting the defect "and again should not be fertilized," say Monk and Holding. At present, the polymerase chain reaction cannot distinguish between the presence of one or two copies of the mutated gene. (Source: New Scientist, 5 May 1990)

Cephalon finds Alzheimer plaque enzyme, synthesizes inhibitors

After isolating the enzyme that causes formation of plaque in the brains of Alzheimer's-disease patients, Cephalon, Inc. has designed inhibitors to it, which it plans to clinically test within two years. Cephalon senior scientist Robert G. Siman has purified to homogeneity the "clipsin" enzyme (Chymotripsin-Like Protease activity) that cleaves the amyloid precursor to the beta-amyloid peptide that makes up a major component of Alzheimer's plaque. Siman and Harvard Medical School co-worker Robert B. Nelson found clipsin was three times more active in the brains of Alzheimer's victims than in normal neural tissue.

Based on a partial "absolutely unique" amino-acid sequence of clipsin, Cephalon scientists have biosynthesized peptide inhibitors to the enzyme, which are effective in vitro at concentrations as low as 10 nM. (Source: McGraw-Hill's Biotechnology Newswatch, 19 March 1990)

Protein isolated that prevents inflammation

Synergen has isolated a protein that can prevent inflammation. The inhibitor might eventually be developed as an anti-inflammatory drug. The protein binds to interleukin-1 receptors. If IL-1 binds to the receptor, a series of reactions are triggered, causing inflammation of the surrounding tissue. This is a factor in rheumatoid arthritis and organ transplant rejection. The IL-1 receptor blocker was isolated from white blood cells. Decoding the protein's structure has allowed implanting the proper genetic sequence in bacteria to produce the protein. Tests in rats show that the compound prevents swelling and inhibits cartilage degeneration resulting from injury-induced arthritis. The compound produced no toxic reactions or clinically significant immune suppression. Human clinical trials will begin by end-1990. Even if the compound itself is not clinically useful, it should help with the development of other anti-inflammatory drugs. (Extracted from Science News, 27 January 1990)

Insulin gene identified

A French research team has identified the structure of a gene sequence which contributes to the development of diabetes. The team from Institut National de la Santé et de la Récherche Medicale (INSERM) claims that post-natal diagnosis of the insulin diabetes is now possible.

The condition, also called juvenile diabetes, is one of the most frequent endocrine diseases affecting children. Its main feature is atropny of the pancreas.

Studying some 50 diabetics and 73 non-diabetics, INSERM identified the gene responsible for sensitivity to diabetes, using DNA amplification technology. The identification could be important for prevention or cures of the disease and suggest ways of identifying genes responsible for other auto-immune conditions. (Source: European Chemical News, 7 May 1990)

Glutamate receptor gene cloned

Scientists at the Jonas Institute in La Jolla, California, have recently identified the gene which instructs the brain cells to synthesize a protein called glutamate receptor. The scientists hope the discovery can lead to a method of reducing the damage caused by glutamate overstimulation during strokes. Glutamate is the main chemical mediator in the brain and is believed to influence epilepsy seizures, strokes, learning and memory. In strokes and severe epilepsy, damaged cells release glutamate in such profusion that it excites neighbouring cells to self-destruct. (Source: European Chemical News, 23 April 1990)

Cancer gene

The discovery of a mutant gene in lung tumours could offer new possibilities for treating lung cancer.

Scientists have long suspected that some people have a genetic susceptibility to lung cancer. Now, a team from the Imperial Cancer Research Fund and the John Radcliffe Hospital in Oxford have found high levels of a mutant form of the so-called p53 gene in cells from lung cancers. The p53 gene is normally a tumour suppressor. The mutant appears to be absent from normal cells or other types of tumour cells.

The team believes the findings make possible "new therapeutic approaches" to lung cancer. (Source: New Scientist, 31 March 1990)

Genetic link to alcoh ism

Researchers at the University of Texas Health Science Center at San Antonio (UTHSCSA) and at the University of California at Los Angeles are the first to demonstrate a specific genetic predisposition to alcoholism as reported in the Journal of the American Medical Association. The presence of a gene that serves as a receptor for dopamine - a chemical in the nervous system - was found in 77 per cent of brain tissue samples from cadavers of alcoholics. This gene, D2 Dopamine, was found in only 18 per cent of tissue samples collected from non-alcoholics. The researchers stress that not everyone with this genetic pattern is a candidate for alcoholism; and that other genes and environment may play roles in this disease. For more information, contact Ken Slavin, or Deedee Donohue, Dublin-McCarter & Associates, 512/227-0221. (Source: BioBytes San Antonio Biotechnology News & Information, April 1990)

The growth of hope

Scientists at the University of Zurich have found a way to make severed nerves in the brain and spinal cord grow again. Although most other nerves regenerate naturally, all previous attempts to make the nerves of the central nervous system grow again have failed. The Swiss technique has so far been demonstrated only in animals, and the regrown nerves have not yet been shown to be fully functional. But if all goes well similar techniques may be used in perhaps seven or eight years' time to help people paralysed below the neck or waist to walk again.

Dr. Martin Schwab and Dr. Lisa Schnell of the Institute of Brain Research at Zurich, succeeded where many others failed by trying a new approach. Others have identified nerve-growth factors, which stimulate the growth of peripheral nerves, and have used them to try to make severed spinal nerves regenerate. Dr. Schwab and Dr. Schnell discovered that the reason why this did not work is that, as well as natural growth-stimulating factors, there are also growth inhibitors in the spinal cord. These prevent regeneration even when growth factors are added.

Dr. Schwab and Dr. Schnell made antibodies against the two nerve-growth blocking factors they found, using the monoclonal technique in which the antibodies are produced artificially in laboratory cell cultures. Then they cut nerves in the spinal cords of rats and grafted living cells from the cultures producing the antibodies into the site of the injury. The cut nerves grew again rapidly, to at least half the length of the rat's spinal cord, 11 millimetres. They found that the nerve-growth inhibiting factors are produced by cells surrounding nerve fibres, called oligodendrocytes. When these cells were heavily irradiated so they could not produce inhibitory substances, nerves regenerated even more rapidly than when the antibodies were used.

It will be a while before similar techniques can be tested on people. First it has to be shown that the new nerves work properly. Then it has to be shown that they grow in exactly the right places. Work on other species suggests that they will. In fish and amphibia severed spinal nerves do regenerate normally. It seems likely that mammals' nerves also have the capacity to do so, but that it has been masked by the inhibitors.

The Swiss team is now investigating the possibility of using molecules smaller than complete antibodies in the hope that they would block growth inhibitors but be easier to use in treatment. They are also planning to combine the use of antibodies or other blockers with growth-stimulating substances, and "bridges" of tissue implanted to help regenerating nerves grow to the correct targets. Dr. Schwab thinks there may be up to 10 years to go before doctors could use such treatments with confidence. But the hope of helping some people out of their wheelchairs is there. (Source: The Economist, 17 February 1990)

Research on animal genes

Transgenic mice to test potentially carcinogenic substances

Researchers at the TNO Institute for Experimental Gerontology (the Netherlands) recently made a transgenic mouse which has a bacterial gene (lacZ gene from the E. coli bacteria) in all its cells integrated on a certain chromosome. The bacterial gene is introduced into the nucleus of a fertilized egg cell by microinjection. This injected cell is then implanted in a pseudo-pregnant mouse (a mouse which has mated with a sterile

male). The offspring will have a fragment of the foreign DNA in all their cells, including the reproductive cells. The foreign fragment will therefore also be passed on to future generations. The lacZ gene is situated in a so-called shuttle vector and can easily be recovered and transferred to bacteria.

One of the properties of the lacZ gene is that it transforms a certain substrate substance, called X-gal into a blue substance. So, if X-gal is added to the bacteria's nutrient medium, this discolouration will point to the lacZ gene being present. However, when the manipulated genetic material of the mouse has been exposed to a carcinogenic substance, mutations will have taken place in the mouse DNA and therefore also in the bacterial gene in that DNA. This is manifested by the fact that after the lacZ has been recovered and added to a bacterial culture, there is no blue discolouration of X-gal. This can easily be determined visually: the small patches of lysed bacteria (plaques) do not turn blue, but remain colourless.

To test the effectiveness of the new method, the transgenic mice were exposed to different doses of the carcinogenic substance ethyl nitroso urea (ENU). After returning the bacterial gene to the bacterial cultures, it appeared that colourless plaques did indeed develop in the animals treated with ENU, varying from 1-3 per 100,000 plaques for the smallest ENU dose to approximately 10 for the largest dose. From the reference animals only blue-coloured plaques were obtained.

The transgenic mice offer great advantages for carcinogenic research. It only requires a few animals, because the frequency of the mutations in each treated animal is approximately the same. Consequently, there is a minimum chance of overlooking a certain carcinogen because it did not cause any mutations. It is therefore not necessary to use many experimental animals and/or large doses of substances to be tested for carcinogenicity.

The TNO researchers believe that the application of the transgenic mouse model will in the long run contribute to a large reduction in the use of experimental animals. However, before that stage is reached, the reliability of this method will still have to be tested thoroughly using a large number of known carcinogenic as well as non-carcinogenic substances.

TNO has reached an agreement with Hazleton Laboratories Corporation, USA, giving Hazleton Laboratories the exclusive rights to commercialize the transgenic mouse model. (Source: Applied Research, No. 28, December 1989)

Cloned lambs

The quest to produce the perfect sheep has moved a step closer with the birth of the world's first cloned merino lambs in the Adelaide Hills, Australia. Researchers from the sheep industry and Adelaide University have developed a technique known as nuclear transfer, allowing 16 cloned lambs to be produced from a single fertilized egg. Adelaide-based Australian Sheep Artificial Breeders (ASAB) has linked up with farmer-businessman Ian McLachan, and Haddon Rig stud at Warren, New South Wales, to further develop the technique. Together they have established the biotechnology firm Emtech Pty Ltd. The company believes that cloning could be commercially viable within two years, following the success of the initial trial. This involved implanting 20 ewes and resulted in the birth of two non-identical cloned lambs from separate experiments. A further 40 ewes have since been implanted. Nuclear transfer involves isolating the cells of an embryo at the 16-cell stage and placing them into unfertilized eggs which have had their nuclei removed. The result should be 16 identical embryos ready for implanting into recipient ewes. Details from: Dr. Alan Cotton, managing director, Australian Sheep Artificial Breeders, 288 Melbourne Street, North Adelaide, South Australia 5006. (Source: Biotechnology Bulletin, Vol. 9, No. 3, April 1990)

Naked mole rats keep it in the family

Genetic fingerprinting has shown that members of a naked mole rat colony are so similar genetically that it is as if brother mated with sister for 60 generations. Naked mole rats are rodents that live very much like the social insects. Zoologists have long known that their colonies are composed of close relatives, but the degree of genetic uniformity has surprised them.

Two teams of researchers hav been studying the genetic material of naked mole rats. Hudson Kern Reeve and his colleagues at Cornell University, New York State, and Christopher Faulkes and his colleagues at the Institute of Zoology in London have independently discovered the amazing uniformity of naked mole rats. The discovery sheds important light on how the society of naked mole rats could have evolved.

Both research groups subjected naked mole rats to genetic fingerprinting. The technique reveals individual idiosyncrasies in the structure of regions of DNA known as minisatellites.

Reeve and his colleagues at Cornell found that naked mole rats from a single colony have almost identical genetic fingerprints. This is very unusual for a population of mammals. Rodents, such as mice, could achieve such uniformity only if they inbred for 60 generations. A colony of naked mole rats is, therefore, very much like a clone.

Faulkes and his team at the Institute of Zoology also took genetic fingerprints. But, in addition, they looked for a variation in a diverse cluster of genes known as the major histocompatibility complex (MHC). This is a component of the immune system. In many species of mammal, individuals who are not related will have a different genetic signature written in their MHC. However, Faulkes and his colleagues found that within a colony of naked mole rats, all signatures are similar. Members of different colonies from the same geographical area are slightly different, but the genetic resemblance remains strong, suggesting a recent common ancestry.

These findings go a long way towards explaining the extraordinary social life of the mole rat, which lives in colonies of 100 or so beneath the sun-baked soils of East Africa. Breeding in the colonies is entirely the preserve of a single queen and one or two consorts - a mammalian equivalent of the royal castes of insect societies. The remaining mole rats in the burrow suppress their reproductive urges and instead spend their time digging new tunnels, defending the colony against snakes and caring for the queen's offspring. These are often their own baby brothers and sisters.

The success of the naked mole rat gives the lie to the traditional notion that inbreeding is always undesirable. As a result of the inbreeding, each member of a colony carries the same highly adapted combination of genes - a state of affairs that seems very desirable. Yet there may be a flaw in the mole rat's approach. If disease strikes, for example, or environmental conditions alter, all inmates of the colony are affected equally. Genetic variation is the raw material of evolution, after all. Without it, a population is condemned to stability in an ever-changing world. (Source: New Scientist, 12 May 1990)

Drugs industry turns animals into "bioreactors"

Animals genetically engineered to become living factories that produce useful drugs or proteins in their milk may soon become the tools of a new industry. Researchers around the world, in universities and in industry, are claiming to have produced valuable pharmaceuticals in animals in commercially viable amounts.

One company, Transgenic Sciences, a life-sciences firm based in Massachusetts, said last week that it had altered mice so that they secrete human growth hormone in their milk at levels of up to half a gram per litre. The company claims that its mice, developed in collaboration with researchers at the University of Massachusetts, show no adverse side-effects at all.

Scientists at the company say they now aim to scale up the techniques they have used on mice, and apply them to rabbits to develop them as "commercial bioreactors". The company estimates that in just three years it will have reached a stage where it is producing human growth hormone in rabbits at a third of the cost of the bacterial cultures used today, and in sufficient quantities to allow laboratory trials. The company hopes to have gained approval for the process from the US Food and Drug Administration by 1996.

John Clark, a leading researcher in this field based at the Institute of Animal Physiology and Genetics Research in Edinburgh, has been working with a local company called Pharmaceutical Proteins, with a view to commercializing his research using genetically manipulated sheep as "bioreactors".

Recently, the company tested a lamb grown from an embryo injected with a fragment of DNA that codes for an antitrypsin, a chemical which can help in the treatment of lung diseases. They found that the gene has been successfully incorporated into the lamb's genetic material. By the time the lamb is lactating next year, they will know whether it can produce the drug in its milk, and at what yield. The company is confident that the sheep will produce high enough yields to be commercially viable, and hopes to be selling the drug by 1995.

When the researchers injected the same combination of DNA, or genetic construct, into the embryos of mice, the resulting animals produced encouragingly high yields of up to 8 grams of the chemical per litre of milk. This is some 1,000 times as high as yields produced previously at Clark's institute. The scientists believe this may be because they have included extra sections of DNA, known as introns, into their construct. These were once thought to have very little genetic effect, but their presence now appears to be responsible for an increase in the yields of proteins for which the main gene is coding.

Other researchers have already transferred genes into mice, sheep and pigs which allow their new host to produce substances such as insulin, tissue plasminogen activator (a blood-clotting agent), and factor IX (a substance missing in some haemophiliacs). But in most cases, the amounts produced have been too small to be commercially useful.

Scientists at Transgenic Sciences used a standard technique of biotechnology to inject their fertilized mouse embryos with the human gene that codes for growth hormone, along with the genetic "instructions" for secreting it. The technique, known as "micro-injection", uses tiny needles to insert the foreign genes into the embryos.

The researchers inserted the altered embryos into a female mouse. Once these embryos have been born and grown mature, they mate to produce offspring of their own. As they lactate to feed their pups, they produce the hormone in their milk.

Scientists at the company are not yet sure why their approach does not induce adverse side-effects, but believe it may be because they have targeted the extra gene so that the mice produce the growth hormone only in their mammary glands. The researchers hope to unravel the mechanisms behind this lack of adverse side-effects with the hope of relieving the commercially inefficient effects shown by cattle and pigs treated with growth factor to encourage them to grow faster, or to have a higher ratio of lean meat to fat. These animals often suffer from arthritis and infertility. (Source: New Scientist, 14 April 1990)

Mouse mode brings leukaemia treatment closer

Researchers in California have developed mice whose genetic material carries an inserted fragment of human DNA. The fragment, long thought to be associated with some forms of leukaemia in people, appears to have the same effect in the mice, so confirming a genetic basis for the disease. The researchers say their animal model of leukaemia will make it easier to study the disease and to test potential drugs to treat it.

Scientists have known since the 1960s that people with some forms of leukaemia have specific chromosomal abnormalities that can be detected in their bone marrow. In such people, two chromosomes, 9 and 22, have swapped some of their DNA: a relatively large piece of 22 becomes attached to 9, while a small piece of 9 joins onto 22. This translocation of pieces of the DNA results in the so-called Philadelphia chromosome.

Two forms of leukaemia - chronic myelogenous leukaemia and acute lymphatic leukaemia - have been clearly linked to the Philadelphia chromosome. Scientists now know that more than 98 per cent of patients with chronic myelogenous leukaemia have the chromosome, and it is present in about a quarter of those who have the acute lymphatic form of the disease.

The Philadelphia chromosome behaves differently from many other translocations, because it is not simply a rearrangement of genes. In the process of swapping, a completely new gene is formed, made up of a fragment of DNA from a gene called bcr on the original 22, together with a gene called abl from 9. The composite gene, bcr/abl, codes for a protein called p190.

Until now, no one knew for sure whether the gene was implicated in leukaemia, although the association between the Philadelphia chromosome and the disease was well known. Now Nora Heisterkamp, John Groffen and their colleagues at the Children's Hospital of Los Angeles have studied a group of 10 mice into whose genetic material they had inserted the bcr/abl fragment. Within 58 days of birth, eight of the mice had died or were near to death with acute leukaemia - either in its chronic myelogenous form or as acute lymphatic leukaemia.

The disease was indistinguishable from its human forms, say the researchers; their findings "confirm the association of the Philadelphia chromosome with leukaemia in man". White blood cells could be affected by p190 at an early stage of development, the researchers believe, so leading to the disease. (Source: New Scientist, 14 April 1990)

Spider silk

A gene from the golden orb spider has been implanted in bacteria to produce a high-strength, stretchable fibre for use in parachute line, bulletproof vests and clothing. The spider silk can stretch to 118 per cent of its original length. The protein produced by the engineered bacteria is spun into fibre by researchers at the US Army R & D centre in Natick, MA. The economics of commercial production of the fibre are now being studied. (Extracted from Chemical Week, 7 March 1990)

Research on plant genes

Better rice varieties

Scientists are using genetic engineering to produce better varieties of rice by making existing strains more resistant to disease, drought and pests. A programme to develop better rice varieties is being co-ordinated by the Rockefeller Foundation to co-ordinate efforts worldwide, including in laboratories in third world countries where rice is a staple food. Researchers have for the first time managed to grow whole rice plants from protoplasts, a feat never before accomplished with cereal plants. In a second major breakthrough, scientists have succeeded in inserting foreign DNA into the rice nucleus and that DNA was passed on to the next generation of rice plants. A third major advance has been the analysis of the entire rice genome. Further refinement of the gene map is needed, but S. Tanksley of Cornell University has indicated the probable areas of specific genes on rice's 12 chromosomes. The map can already be used to help with conventional breeding of plants to provide desired characteristics.

Tranh Ton That of FAO says conventional rice genetics has reached a plateau, and further advances will likely come from genetic engineering. Rice provides 20 per cent of the world's food, in terms of calories. The Rockefeller Foundation has already spent $25 million on the programme to improve rice, and now spends about $6 million per year on the project. (Extracted from New York Times, 6 February 1990)

Mouse mabs grown in tobacco plants

Scripps Clinic & Research Foundation (La Jolla, CA) has developed a technology for growing mouse monoclonal antibodies (Mabs) in tobacco plants. Researchers cloned gamma AND kappa immunoglobulin genes from mice. Each gene was injected into different tobacco plants, which were cross-pollinated when mature. In 25 per cent of the tobacco progeny, the two mouse genes combined to make functional Mabs. Mabs created by this process may not cause the immune rejection problems in patients that often occur with normal mouse Mabs treatments. The process may decrease production costs from the current $5,000/g to perhaps 10 cents/g. (Extracted from Genetic Engineering News, January 1990)

Blushing plant cell

It is now easy for genetic engineers to create new varieties of plants by transferring genes from one species to another. But the researchers' task could now become easier. To keep track of the genes in transit, they need to make use of so-called "reporter genes". These are genes that act as markers indicating which cells in the receiving plant have taken up the foreign DNA and are producing proteins from its instructions. Molecular geneticists in the US have found a new type of reporter gene: it causes cells to produce a red pigment which is visible to the naked eye, making the affected cells easy to detect.

Researchers at the University of Georgia at Athens, and at Pioneer Hi-Bred International at Johnston, Iowa, used a gene known as Lc, isolated from maize. It is a member of the R family - genes that control the location, timing and amount of a pigment called anthocyanin that is produced in maize cells.

The team removed the normal sequence of DNA that controls the rest of the gene, and replaced it with another control sequence which would allow the gene to work in all cell types. When they introduced this altered gene into plant cells, these cells produced anthocyanin and each cell turned bright red.

The appeal of the new marker is that researchers can see the red pigment without any further biochemical treatment. If the tissue they are examining is near the surface, they do not need to disturb the plant. Many tests now used to identify genetically altered cells kill them in the process; others do not distinguish so readily between adjacent altered and unaltered cell lines.

The researchers introduced the Lc gene into maize tissues by bombarding them with microprojectiles bearing the gene. As a genetically altered cell replicates in the developing plant, the biologists can watch a red streak grow. (Source: New Scientist, 5 May 1990)

Altered gene in corn passed to next generation

Plant Science Research (Minnetonka, MN) has developed the first genetically engineered corn hybrid that passes the altered gene to the next generation. The company has filed for a patent for its genetic technology and will apply for USDA field-testing permission. The company did not explain its technology or which gene was transferred. According to R. Klees, president of Plant Science Research, genes which may be of interest are those for tryptophan and lysine, which would increase the nutritional value of animal feed corn, and the cow pea trypsin inhibitor gene, which offers insect resistance. Genetic engineering of monocot seeds such as corn has been difficult to achieve while maintaining fertility. If the technology is applicable to other monocots such as wheat and rice, the company will have an advantage in the market. Plant Science Research is a

subsidiary of Biotechnica Intnl (Cambridge, MA). The world market for corn products is estimated to be around $70 billion. (Extracted from Genetic Engineering News, March 1990)

Research on bacterial genes

Bacteria used in "magic bullet" drugs

Genetically modified bacteria are under investigation by a number of companies as a vector for transporting drugs to specific target tissues in the body.

Wellcome Biotech's Dr. Gordon Dougan described at a recent Biochemical Society meeting in the UK how the company's scientists have been able to alter bacteria so that their ability to enter host tissues remains unaffected while their ability to multiply and cause disease is destroyed.

Dougan explained that the genes necessary for proliferation of the bacteria can be removed so that they no longer have pathogenic properties. In addition, genes which encode antigens from other bacteria or for therapeutic proteins can be introduced into the bacteria. In this way, the bacteria are rendered harmless and can be used for the "magic bullet" delivery of drugs.

Some bacteria, such as Salmonella, are particularly useful for delivering products to the immune system cells, which they directly target. This would be useful for products like interleukin-1, according to Dougan.

Altered Salmonella bacteria have also been used to deliver a vaccine for diarrhoea diseases, while altered bacteria, which infect the respiratory tract, have been used against lung diseases such as whooping cough. Wellcome Biotech is using the method to develop an oral tetanus vaccine.

Wellcome's scientists are attempting to identify the proteins on the bacterial cell surfaces which attach the bacteria to the target cells in the host. If the genes that provide a blueprint for these attachment and invasion proteins can be characterized, Dougan believes it may be possible to use them to produce proteins that will deliver the drugs specifically to the cells without using the bacteria. (Source: European Chemical News, 23 April 1990)

Antibody genes cloned in bacteria

The entire repertoire of an animal's antibody genes can be cloned and expressed in bacteria, rather than in hybridoma-cell cultures, reported Richard A. Lerner of the Scripps Clinic and Research Institute. The technology was developed jointly by a group headed by Joseph A. Sorge, CEO of Stratagene Corp., a privately held biotechnology firm.

Stratagene has founded a subsidiary, Stratacyte Corp., to commercialize the antibody technology. Stratacyte plans using the technology to develop therapeutics for cancer, autoimmune and infectious diseases, to refine diagnostic tests and develop enzymes for medical and industrial processes.

The bacteria-produced antibodies will make it possible, says a Stratacyte press release, "to inexpensively screen and produce pure-human monoclonal antibodies, eliminating the problem of harmful immune reactions in patients receiving animal-derived antibodies". In addition, the company states, the bacterial technology bypasses tumour cells and animals in producing antibodies, thus saving time, expense and animal sacrifice. Stratacyte plans to improve the technology to allow screening of trillions of monoclonals instead of millions, thereby increasing the chances of finding rare, valuable monoclonals.

The technique for cloning antibody variable genes directly for expression was initially developed by senior scientist Greg Winter and his colleagues at the Medical Research Council's (MRC) Laboratory of Molecular Biology, Cambridge, UK. They created chimeric antibodies in which the specificity of a rodent antibody was transferred to a human antibody. "Chimeric antibodies are promising for treating a wide range of diseases, from cancer to infections." In 1988, "in the first study to show the clinical effectiveness of chimeric antibodies," Winter added, he and co-workers used such antibodies, produced in animal cells, to induce remission in two patients with non-Hodgkin lymphoma. Winter expects this antibody, currently under evaluation by Wellcome Biotechnology Ltd., Kent, UK, to reach the market by the mid-1990s.

Despite the potential of the new technology, Winter is cautious in his assessment, particularly about the prospects of bypassing animals completely. Another question is how well the mass screening methods, which are a critical feature of the bacterial technology, can be applied to a variety of antigens. Winter sees "a very big field opening up, but success will depend on the availability of good screening methods for the antigen. The limiting step is screening, not the fusion. One of the advantages of expression in bacteria is that it will encourage development of new screening methods." (Source: McGraw-Hill's Biotechnology Newswatch, 15 January 1990)

Bacteria DNA identification system

Shimadzu Corp. has developed a bacteria identification process with gene amplification technology and has developed a clinical system that detects pathological bacteria quickly and very sensitively. This bacteria DNA identification system uses a reagent for directly detecting the DNA of bacterial genes to identify specific types of bacteria.

With the conventional bacteria identification system (culture method), tests are conducted by culturing which takes time, so two to three days at least are required for sampling specimens from the patient and analysing the results. Also, since the results depend largely on skill, the accuracy is rather unstable.

The new system uses a newly developed reagent that binds to a specific DNA sequence from a gene and attaches a marker that fluoresces directly identifying the bacterium strain. The time required is about five hours, and testing is possible even when only about 100 pathological bacteria are present in the specimen, so diagnosis is quick and very sensitive. So far, reagents have been developed for eight kinds of food poisoning bacteria such as salmonella and Welch bacillus.

The system enables early identification of pathological bacteria, and permits optimum selection of drugs such as antibiotic agents at an early stage of the disease. Further details available from:

Shimadzu Corporation
Marketing Research and Planning Dept.
1 Nishinokyo Kuwabara-cho, Nakagyoku
Kyoto
Tel.: 075-832-1111 Fax: 075-811-3188

(Source: JETRO, May 1990)

Nitrogen fixation

Researchers are still trying to ascertain how biological nitrogen fixation works, in an effort to develop new catalysts for ammonia production. The commonly used Haber process to convert atmospheric nitrogen into ammonia requires temperatures of 400-500°C, and pressures of 250 atmospheres. Biological nitrogen fixation occurs at normal temperatures and pressures. Nitrogen fixing bacteria have an enzyme (nitrogenase) that includes iron and molybdenum, which are both transition elements. The initial step of transforming dinitrogen to diazene (H_2N_2) requires a great deal of energy. Instead, biological systems probably use molybdenum to bind to one nitrogen of dinitrogen, allowing the triple bond to the other nitrogen to break, thus freeing the second nitrogen atom to bind to an atom of hydrogen supplied by an acid. The second and third bonds also become relatively easy to break, yielding ammonia. The reactions also generate a new dihydrogen (H_2) molecule.

At least one type of nitrogen fixing bacterium does not use molybdenum-based nitrogenase, but rather a vanadium-based nitrogenase. This produces three extra dihydrogen molecules. Yet a third type of nitrogenase has now been discovered, which contains only iron. The nitrogenases might, in fact, all operate independently of the transition metal that they contain. Further research may soon unravel the mystery of how nitrogen fixation really occurs (Extracted from New Scientist, 10 February 1990)

Research on viral genes

HPV can cause cells to proliferate

Human papillomavirus (HPV) genetic material can cause human breast cells to proliferate indefinitely in vitro, according to researchers at the Dana-Farber Cancer Institution (Boston). This is the first time researchers have had a way to transform healthy breast cells. HPV has the same effect on skin cells. The DNA from HPV types 16 and 18 caused breast cells to grow for two years as they would in a benign tumour. Further studies are planned to determine if the cells can be mutated to make them cancerous. (Extracted from Science News, 27 January 1990)

Various compounds that are specific, potent antiviral agents against the human immunodeficiency virus type 1 have been synthesized by researchers at the Katholieke Universiteit Leuven and the Janssen Research Foundation in Beerse, Belgium, and Spring House, PA. The antiviral agents all belong to a class of compounds known as tetrahydro-imidazo (4, 5, 1-jk) (1, 4)-benzodiazepin-2(1H)-one and -thione derivatives. They can inhibit HIV-1, but have no effect on HIV-2, DNA OR RNA viruses. The new compounds contain a novel tricyclic system in which 5-, 6- and 7-membered rings are joined together. Some of the derivatives can inhibit HIV-1 replication in cell culture at nanomolar concentrations 20,000-30,000 times lower than levels that impair the viability of uninfected human lymphocytes. Zidovudine (AZT) inhibits HIV-1 replication at a level only 6,000 times less than its cytotoxic concentration. The safety margin is smaller yet for the experimental antiviral agents, DDI and DDC. All of the new derivatives have a (+)-(S) configuration at C5, which seems to be necessary for anti-HIV-1 activity. (Extracted with permission from Chemical and Engineering News, 5 February 1990, p.p. 5-6, R. Dagani. Copyright American Chemical Society, 1990)

Chimp virus

Researchers in Paris have finished sequencing the genetic material of an AIDS-like virus isolated from a chimpanzee in Gabon.

Initial studies showed that the virus was very similar to HIV-1, the first virus identified as the cause of AIDS. It is more common than HIV-2, which is found mainly in West Africa.

The details of the virus's genetic sequence will tell scientists how closely related it is to other viruses that attack the immune system in humans and in other primates. (Source: New Scientist, 24 February 1990)

Americans puzzle over new form of hepatitis

American scientists have isolated the virus that causes what is believed to be one of the most lethal forms of hepatitis in the third world. They are now preparing a test for the virus before developing a vaccine. The test may reveal whether hepatitis E is a relatively new disease in humans.

Hepatitis is inflammation of the liver. It can be caused by chemical damage, or by disease-producing organisms. Hepatitis A is an acute infectious disease, caused by a virus spread on eating utensils. Hepatitis B is a chronic viral infection transmitted in blood and other bodily fluids, and is linked with a major cancer, hepatocellular carcinoma. Both are common in the third world and can be identified in simple screening tests.

Hepatitis viruses other than A and B have also been identified. Last year, scientists isolated the hepatitis C virus and the D virus, both blood-borne. Hepatitis E is much more important. It is spread through water contaminated with faeces from infected people. Mark Kane, who works for the World Health Organization in Geneva, estimates that it affects up to a million people each year. In Asia, says Kane, it probably accounts for over half the cases of acute viral hepatitis in adults. Pregnant women are especially vulnerable; a fifth of those who contract the virus die. The exact prevalence of the virus is unknown because there has been no simple way to test for it.

Daniel Bradley, head of hepatitis research for the Centres for Disease Control in Atlanta, says the hepatitis E virus now isolated by his laboratory is larger than the other viruses and is not related to them. It was found in faecal samples from patients in Mexico, Borneo, Somalia, Burma, and Tashkent in the Soviet Union. The virus infected macaques and was present in their gall bladders in large quantities.

The genetic material of virus E has now been sequenced by Genelabs of California. The viral proteins expressed by the cloned genes will be used to develop a simple screening test for hepatitis E. Public health authorities will then be able to track the incidence of infection.

The next step will be a vaccine. Bradley says monkeys immunized with a weakened virus from one geographical location were protected from infection with virus from elsewhere, suggesting that it may be possible to develop a general vaccine.

Hepatitis E primarily affects young adults, however, which may show that it does not induce very effective immunity. Infection in childhood might cause partial immunity, which wears off to permit re-infection in adulthood. This does not necessarily mean a vaccine based on the virus will induce only partial immunity. There are ways of making a vaccine that confers more immunity than its parent organism, says Kane. He says the tendency of hepatitis E to strike young adults may not be a sign of poor immunization; it may mean that the virus is a new disease. If it were new, it would be relatively scarce so that people would not encounter it until adulthood. (Source: New Scientist, 24 March 1990)

Ribozymes cleave AIDS virus genetic material

Catalytic ribonucleic acid molecules, or ribozymes, have been designed to cleave, sequence specifically, the RNA of the human immunodeficiency virus (HIV). The work could lead to use of ribozymes as therapeutic agents against HIV, the virus that causes AIDS, or eventually in anti-HIV gene therapy.

The research was carried out by Nava Sarver, section chief in the developmental therapeutics branch of the National Institute of Allergy & Infectious Diseases' AIDS branch in Bethesda, Md., working with John J. Rossi, John A. Zaia, and co-workers at the City of Hope Medical Centre, Duarte, California.

Sarver says Rossi is experimenting with mechanisms for delivering the ribozymes as therapeutics. Their most potent application might be in gene therapy, in which an AIDS patient would receive bone marrow cells engineered to express the ribozyme. Such gene therapy, however, is at least several years away. (Extracted with permission from Chemical Engineering News, 12 March 1990, p.6, R. Baum. Copyright American Chemical Society, 1990)

Cell protein enhances AIDS-like infection

Scientists at Southwest Foundation for Biomedical Research in San Antonio have shown that CD4, a protein on the surface of human cells, plays an integral role in the AIDS infection process. This genetically engineered protein, currently being used in clinical trials as a therapy for AIDS patients, actually enhances infection of a related AIDS virus found in monkeys. In addition to using CD4 as a primary receptor for infection, these AIDS viruses can infect white blood cells through secondary sources. This discovery is expected to provide better understanding of how viruses penetrate and kill human cells and will hopefully lead to the development of an effective AIDS vaccine targeted specifically to these secondary events in viral infections of immune cells.

For more information, contact Ken Slavin or Dee Dee Donohue, Dublin-McCarter & Associates - 512/227-0221. (Source: BioBytes San Antonio Biotechnology News & Information, March 1990)

Research instrumentation

Discrimination of immature leukocytes with neural network

Hitachi, Ltd. has developed a technique for discriminating mature and immature leukocytes with a high degree of accuracy by using a neural network.

Leukocytes in human bone marrow and other tissue consist of more than 20 different kinds for distribution into the peripheral blood. The leukocytes remaining in the hematogenous bone marrow and other tissue are known as immature leukocytes and those distributed into the peripheral blood are known as mature leukocytes.

In a healthy person, the distribution ratio of various leukocytes in the peripheral blood is within a fixed range; and when a change occurs in this distribution ratio, they may be suffering from some disease. Much skill is required for examining the distribution of the different leukocytes and judging whether there are immature leukocytes in the blood, and such examinations are exhausting.

The company conceived the idea of using the neural network, which is known to be effective in pattern recognition, and succeeded in developing the new technique.

With this new technique, a blood smear is dried and stained, the leukocytes are magnified with a microscope, input into a TV camera, then digitally converted. Next, the leukocyte nuclei and 13 cytoplasm characteristics (such as area, circumferential length, colour tone, and existence of granules) are extracted to discriminate mature and immature leukocytes. The leukocytes in blood can be classified into 30 different kinds: 7 kinds of mature leukocytes, 18 kinds of immature leukocytes, and 5 kinds of artifacts (destroyed leukocytes, residual stain substances, etc.).

The neural network serving as the system's arithmetical unit incorporates a back propagation function for repeatedly acquiring learning to gradually minimize the error ratio. Evaluation with a simulator showed an immature leukocyte detection ratio of 91.5 per cent, previously 83.7 per cent, and accurate discrimination of mature and immature leukocytes. Further details available from: Hitachi, Ltd., Public Relations Secretary's Office, 4-6 Kanda Surugadai, Chiyoda-ku, Tokyo. Tel.: 03-258-1111 Fax: 03-258-5480 (Source: JETRO, May 1990)

Heat shock stress protein probes

CBR International Biotechnologies Corp. (B.C., Canada and California, USA) offers a wide selection of DNA probes and monoclonal antibodies specific for the individual heat shock (stress) proteins (HSP), some of which have a broad range of species cross reactivity. Purified antibodies against the 90kDa protein and the inducible and constitutive forms of the 70kDa proteins are supplied lyophilized. The DNA probes are plasmid-based with inserts specific for the 27kDa, 60kDa, 70kDa (inducible), 70kDa (constitutive), and two variants of the 90kDa mRNA. The reagents are available individually for research purposes or discounted when purchased in quantity or as the complete set of monoclonal antibodies or DNA probes. The company will be releasing additional HSP-based research tools against other stress proteins and an array of HSP-based expression

vectors later this year. For additional information, contact CBR International at PO Box 2010, 9865 W. Saanich Rd, Sidney, BC, Canada V8L 3S3. (Source: ABA Bulletin, Vol. 5, No. 2, April 1990)

New monoclonal oncoprotein antibodies for research and potential diagnostic use

Utilizing expertise in the synthetic peptide approach to antibody production, Cambridge Research Biochemicals has produced a range of murine hybridomas secreting antibodies capable of specifically-targetting epitopes contained within the cytoplasmic and external domains of both human EGF-R and c-erbB-2, and from the nucleus-associated myc oncoprotein family. These highly specific and sensitive antibodies have been characterized for native protein reactivity by immunoblotting, flow cytometry, immunoprecipitation and immunocytochemistry. In view of the perceived importance of reagents specific for EGF-R, c-erbB-2 and myc proteins in the diagnosis and prognostic assessment of various abnormal physiological states, the antibodies available from CRB may prove to be of considerable utility and value to those involved in clinical, diagnostic and therapeutic research.

The hybridomas are available for licensing or purchase and the antibodies form part of a range of over 80 oncoprotein-derived antibodies, peptides and kits produced by CRB. Cambridge Research Biochemicals Ltd. is wholly owned by Imperial Chemical Industries (ICI) plc and is part of the ICI Biological Products business. In addition to the range of oncoprotein antibodies CRB also offers an extensive range of peptide antibodies, conjugation kits, synthetic haptens and epitope scanning kits suitable for use in immunology research. For further information contact: Dr. Ian Varndell, Cambridge Research Biochemicals Ltd., Buttend End, Harston, Cambridge CB2 5NX, UK. (Source: ABA Bulletin, Vol. 5, No. 2, April 1990)

Bacterial DNA identification kits

Shimadzu has developed a technique for identifying bacterial DNA in five hours using gene amplification technology. Specific reagents will be used to detect the DNA from pathogenic bacteria and label it with a fluorescent marker. Reagents are available for eight pathogenic bacteria. The new kits can identify bacteria in just five hours, rather than the two to three days now needed for culturing. (Extracted from Japan Chemistry, 8 February 1990)

Versatile controlled-release device for pheromones

Researchers of the TNO Centre for Polymeric Materials (the Netherlands) have developed a new type of dispenser for pheromones. Major advantages of the dispenser are the greater versatility compared with other controlled-release devices and the excellent protection against chemical degradation of pheromones.

Pheromones are chemical compounds used in intraspecific communication between animals. Well-known examples are the sex pheromones secreted by females to attract males for mating, trail pheromones and alarm pheromones.

By identifying and synthesizing these substances, one can use them in environmental-friendly techniques for insect pest control. For instance, traps baited with pheromones are used to catch a large number of males, leading to a decrease in reproduction. This method is known as mass trapping. A decreased reproduction rate can also be achieved by the so-called "male confusion technique": the atmosphere is permeated with pheromone with the aim of preventing the males from locating the females. Traps baited with pheromones can also be used for determining the presence, location and degree of infestation, enabling accurate timing of spraying insecticides. Finally, using pheromone-insectide combinations, one can minimize the amount of insecticide, owing to the fact that insects are attracted to pheromone-baited release systems that have been treated with an insecticide.

To maintain an effective concentration of pheromones in air for a long time, it is necessary to have a controlled-release device that prolongs the biologically active period in the field.

TNO researchers have developed a new type of controlled release system that has greater versatility compared with other controlled-release devices. The release rate can easily be adjusted to local field conditions by changing the polymeric formulation. For instance, Codlemone is formulated in a two-component system. The release rate can be varied by changing the fraction of these components in the formulation. The shape of the dispenser material can also be varied (granulate, rigid blocks, films, coatings). This new technique enables almost any pheromone to be formulated.

The longer the desired field life of the pheromones, the larger the quantity of pheromones that will be degraded (e.g. by oxidation and isomerization of double bonds). Not only does this lead to loss of active substance, newly formed compounds sometimes also have adverse effects on pheromones.

In the TNO dispenser several kinds of substances can be added to the formulations to protect the pheromone. TNO researchers have studied the effect of stabilizers (antioxidants, UV absorbers, etc.) on pheromone stability under both laboratory (accelerated aging tests) and field conditions. In this way they have developed a formulation with excellent protection against chemical degradation of these senstiive biologically active chemicals. TNO has filed a patent application for this new type of pheromone formulation.

The researchers developed a controlled-release formulation for Codlemone (E,E-8,10-dodecadienyl alcohol), the sex pheromone of the fruit moth Laspeyseria pomonella. Field studies carried out by the Research Institute for Plant Protection together with TNO showed that the release rate decreased to only 50 per cent of the initial release rate over a period of four months. Analysis of the remaining material did not point to any chemical conversion of the formulated pheromone during this period.

Besides controlled-release systems for pheromones, the TNO Centre for Polymeric Materials also works on formulations for other biological insect control agents (viruses, repellents), pharmaceuticals and pesticides. (Source: Applied Research, December 1989/28)

Automass 1000 revolutionizes affinity separation

NYGene Corp. has introduced the AutoMASS 1000, a completely automated membrane affinity separation system that drastically reduces the time and costs of purifying monoclonal antibodies and other biopharmaceuticals used to detect, diagnose, and treat cancer, AIDS, and other diseases. Since their development in the mid-1970s, monoclonal antibodies and other biologicals - new classes of medical weapons derived from the body's immune system and genetic engineering - have been heralded as revolutionary new approaches to medicine. But this revolution has been slowed because, until now, purifying biologicals has been essentially an art: The use of HPLC and gel columns (the standard separation technologies), is costly, labour intensive, lengthy, and requires highly trained personnel. For example, it can take a senior scientist using standard technologies an entire day to purify just 10 grams of pure monoclonal antibody.

The NYGene AutoMASS 1000, the first-ever flat membrane separation instrument system, has transformed this labour-intensive, time-consuming task into a fast, easy, and reproducible scientific method that radically improves the economics of monoclonal antibody production. The coupling of AutoMASS's computer-controlled instrument with the core technology of NYGene's MASS (Membrane Affinity Separation System) devices offers researchers and industrial manufacturers of biologicals unprecedented product recovery rates, process and purification cycle speeds, and ease of use. Now, using the AutoMASS 1000, one technician can reliably process and purify 250 grams of monoclonal antibodies a day - an efficiency improvement of 2,500 per cent over the standard technologies.

At the heart of the AutoMASS 1000 is NYGene's MASS^{R} device. Introduced in January 1988, MASS^{R} devices have reliably delivered yields of 85-95 per cent and purities of 98 per cent + in hundreds of research and commercial laboratories in the US and abroad. MASS^{R} achieves these results in a single four minute pass of solution through the device. (In comparison, the next-best separation technology takes several hours to produce 50-80 per cent yields.) Protein A, Protein G, and Universal Affinity Membrane (available with a wide variety of chemistries useful to purify interleukins, interferons, growth factors and other biopharmaceuticals) MASS^{R} devices are sold in sizes ranging from 1 mg to 1 gram and larger. Each MASS^{R} device is supplied ready for use, eliminating the elaborate set-up and time-consuming training associated with most competitive separation technologies. Any MASS^{R} device (including MASS^{R} devices with chemistries yet to be developed) can be easily clamped into the AutoMASS 1000, giving NyGene customers the ability to change chemistries or to scale-up their production capabilities without having to change equipment or methodologies. (Source: Company News Release, 2 April 1990)

Non-isotopic oligonucleotide labelling kits

Two new oligonucleotide labelling kits, E-LINK^{TM} and E-LINK PLUS^{TM} have been launched by Cambridge Research Biochemicals Ltd.

Each kit provides all the reagents, and a simple protocol, needed to covalently label two oligonucleotides with alkaline phosphatase.

The E-LINK PLUS^{TM} kit contains Lumi-Phos^{TM}, a stable, highly sensitive chemiluminescent substrate, which produces a signal on X-ray film, with results shown to be equivalent to, or better than, ^{32}P. The E-LINK^{TM} oligonucleotide labelling kit contains no chemiluminescent substrate and allows the user to be flexible in their choice of substrate.

Researchers without any chemistry knowledge can perform the conjugation with ease. The procedure takes about one hour hands-on time, including a simple chromatographic step using a pre-packed column provided in the kit which gives a purified conjugate ensuring optimal performance. Covalent linkage reduces the background signals often associated with indirect labelling procedures and simplifies most applications. The protocol includes applications notes for Southern and Northern blotting, dot blots, plaque lifts and in situ hybridization. The kits are available from:

Cambridge Research Biochemicals Ltd.
Gadbrook Park
Northwich
Cheshire CW9 7RA
England

Cambridge Research Biochemicals Inc.
Wilmington
DE 19897
USA

(Source: Company News Release, 29 March 1990)

General

Population "bottlenecks" enhance a species' genetic resources

Species that squeeze through so-called population bottlenecks may be better equipped genetically to respond to environmental changes, and are not impoverished as the standard biology texts state. This has important implications for understanding how new species might arise under natural conditions.

A bottleneck develops when a population crashes because of some catastrophe, and then recovers. According to traditional theory, the process depletes much of the genetic variation in the population, leaving the recovering population with less genetic equipment with which to adapt to new environments. Hampton Carson and Robert Wisotzkey, of the University of Hawaii claim that this is not always so. They found that in fruit flies genetic variation can increase following a bottleneck.

Their observations strengthen similar conclusions reached recently by Edwin Bryant and his colleagues at the University of Houston, Texas, who worked with houseflies.

For more than 30 years the genetics of small and large populations has figured prominently in discussions about the origin of species, with bottlenecks always a conspicuous feature. The question at issue is: Are large populations more likely to make the important genetic shift towards speciation than small populations?

In the 1950s, Ernst Mayr, at Harvard University, suggested that there is too much "genetic inertia" in large populations for speciation to occur easily. New mutations - the driving force of change - would be swamped in the population mix, he said. But, if the population crashed, or if a small sub-population became

genetically isolated from the parent population, Mayr speculated, then the genetic variation in the small population would be released from its inertia, and speciation might result. That was one side of the argument.

On the other side, it was said that large populations are more likely to undergo genetic change towards speciation, because they contain more genetic variation as a whole. The population has a greater likelihood of responding to changes in the environment. Moreover, if you pluck just a few individuals from such a population, which is effectively what happens in a bottleneck, they will carry with them only a small part of the overall genetic variation. So the small population has diminished genetic resources with which to make an evolutionary leap when the situation demands it.

The position that small populations inevitably have impoverished genetic resources has become embedded in population genetic theory. "I think the reason has to do with the limited power of models when faced with the great complexity of the real world," says Bryant. "In making models of biological systems researchers are forced to simplify, and in simplifying out the complexity you sometimes distort the outcome," he says.

The potential complexity of genetic systems makes the mathematics extremely complicated. The reason is that genetic variance comes in several forms, the behaviour of some of which is only little understood.

The increase in genetic variance appears, in part at least, to result from a greater prominence of rare, recessive and sometimes harmful variants of genes (alleles) in the new population. (Source: New Scientist, 10 February 1990)

Are molecular clocks slowing down?

Of all the taxonomic groups that have come under the scrutiny of molecular clocks, the primates have received most publicity. And it is among the proponents of these molecular clocks that the idea of slowdown has been debated most intensively. "You see slowdown wherever you look," said Morris Goodman of Wayne State University, "but it looks really interesting in the higher primates."

In the face of counter arguments from his critics at the University of California at Berkeley, Goodman has long argued that a reduction in the rate of the accumulation of mutations over time was inevitable. "In essence, the slowdown hypothesis proposed that over eons of time natural selection increased the internal complexity of life and, in safeguarding the new and complex functions that had evolved, slowed the rate of molecular evolution," explained Goodman. From time to time, however, the pressure of natural selection shifts as organisms experience new environments, and the rate of mutation may increase dramatically for a while. But, overall, there will be a general drift towards lower rates through time.

Here, then, Goodman is arguing for a gradually reduced rate of mutation in the protein structure of the organism through evolutionary time. But, he says, the phenomenon goes deeper, to the level of the DNA sequence, even to sequences that do not directly code for amino acids. "The key premise is that a majority of genomic DNA sequence changes are neutral changes having little or no effect on the phenotype," explains Goodman. "Thus, decreases in de novo mutation rates should decrease rates of DNA sequence change."

But why should rates of new mutation decrease through evolutionary time? The main reason is a fine-tuning of biochemical repair mechanisms that guard the fidelity of DNA replication. But another factor is difference in generation time in large and small species. For instance, for every human generation, mice run through 100 generations. And, as the production of each generation is an opportunity for mistakes to occur in DNA replication, you expect to see a much higher rate of mutation in mice. There is, but it is only a five-fold difference, not 100-fold.

The reason that the disparity is smaller than predicted is that it is in the turnover of germline cells that replication errors are accumulated, not simply at each new generation. "There is a correlation between generation time and germline turnover, but it is clearly not a direct relationship," said Goodman. From this, you would expect the small-bodied (short generation time) primates to have a higher rate of mutation than the large-bodied primates (long generation time). As primates have tended to grow larger through evolutionary time, the lower mutation rate associated with these species would also be reflected in the group's history.

By now Goodman and his colleagues have accumulated considerable sequence data about the primates, particularly data from various globin genes. In primates as a whole, the data show a drop in the rate of non-coding changes through time. In addition, says Goodman, the studies "clearly demonstrate that marked nonuniformities in the accumulation of mutations ... have occurred in different primate lineages," said Goodman. Rates among the small prosimian primates such as tarsiers and galagos are highest, with New and Old World monkeys clocking in about half this rate, and apes and humans about half the monkey rate. Completing the trend, the greatest slowdown seems to be among the human ancestors, the hominids. (Source: New Scientist, 10 February 1990)

Smoking damages DNA in cervical cells

Doctors have known for a long time that women who smoke increase their risk of developing cervical cancer. But the link has been unclear. Now a group of researchers has demonstrated that smoking damages the DNA in cells of the cervix, the neck of the womb.

Penelope Ward of St. Mary's Hospital Medical School, London, and her colleagues looked at the tissue of women undergoing routine cervical smear tests. They reported their findings at the annual meeting of the British Society for Colposcopy and Cervical Pathology, held in Sheffield.

The researchers suspected cervical cells might be damaged because previous studies have found the constituents of cigarette smoke in the mucus which coats them. Smoking could damage DNA in several ways. One way would be for it to cause DNA "adducts" - extra compounds - to form on the genetic material of the cell. These adducts are products of the aromatic hydrocarbons in smoke which bind to DNA. They are an early step in the development of cancer.

Previously, researchers have detected adducts in the DNA of cells of the lungs and placental tissue of smokers. Those who smoked the most showed the most damage.

Ward and her colleagues studied 22 women. They measured the quantities of DNA adducts in tissue obtained from cervical smears. Thirteen of the women were currently smoking; nine had never smoked.

The non-smokers had no adducts or adducts at low levels only. Of the smokers, two thirds had levels of adducts in the same range as the non-smokers, but three of the smokers had very high levels of adducts indeed. Ward and her colleagues now want to know whether these smokers are a subgroup of smokers specially at risk of developing cancer, or precancerous changes, to the cervix. To test this, they now hope to look at adduct levels in patients with established invasive cervical cancer. (Source: New Scientist, 14 April 1990)

Advance in site-specific DNA recognition

In yet another step towards a general chemical solution to the problem of sequence-specific DNA recognition, chemists at California Institute of Technology have designed oligonucleotides capable of binding alternate strands of DNA by triple-helix formation. Caltech chemistry professor Peter B. Dervan and colleagues had shown previously that an oligonucleotide containing pyrimidines will form a triple helix with homopurine-homopyrimidine local tracts within large duplex DNA by binding to the purines in the major groove of the nucleic acid. Dervan and postdoctoral fellow David A. Horne have now shown that the incorporation of a-3'-3'phosphodiester and a 1,2 dideoxy-D-ribose linker permits construction of a pyrimidine oligonucleotide that binds to a stretch of purines on one strand of the DNA double helix and then crosses over the major groove to bind to a stretch of purines on the opposite side of the major groove. This ability to cross from one side of the major groove to the other overcomes in part a limitation on triple-helix formation by pyrimidine oligonucleotides as a general strategy for sequence-specific DNA recognition. Chemists may now target a much more diverse set of DNA sequences. (Reprinted with permission from Chemical and Engineering News, 26 March 1990, p. 14. Copyright American Chemical Society)

How yeast cells know when to divide

What makes a cell divide? Until now, biologists have not known what tells a growing cell that it must undergo the process of mitosis and split into two daughter cells. A team of researchers from Britain and the US believes it has the answer. It has identified a protein that plays a key role in telling a cell that it has grown enough to split in two.

Sergio Moreno and his colleagues at the University of Oxford and Scripps Clinic in La Jolla, California, have studied cultures of the yeast, Schizosaccaromyces pombe. The cells of this organism are cylindrical and they grow by elongating their tips before dividing in the middle.

The researchers noted that cells of S. pombe that have a mutation in a gene known as cdc25 are unable to divide in mitosis. The gene codes for a protein called $p80^{cdc25}$. The biologists have found that as S. pombe cells grow, the protein accumulates, together with its messenger RNA. The two substances reach their highest concentration as the cell begins to split in the process of mitosis.

Moreno and his colleagues propose that mitosis is initiated in a cell when a critical level of $p80^{cdc25}$ has accumulated. In this way, they say, the protein causes a cell to divide only when it reaches a critical size. According to the researchers, if a cell synthesizes the protein at a different rate, it will undergo mitosis when it has reached a different size from normal. For example, if the protein is produced at a greater rate, this results in smaller cells. In this way, cell division is co-ordinated with cell size.

The researchers still do not understand precisely how $p80^{cdc25}$ acts at the molecular level, but it appears to activate a "protein kinase" enzyme known as $p34^{cdc2}$. The enzyme adds a phosphate to the other proteins, and so regulates key proteins which are involved in mitosis.

Biologists have found protein kinases analogous to the yeast $p34^{cdc2}$ in the Xenopus, a toad, in starfish, and also in humans. For this reason, they believe that the enzyme is part of a universal control of mitosis which is common to all eukaryotic cells, cells which have a distinct nucleus in them. (Source: New Scientist, 12 May 1990)

DNA fingerprinting questioned in criminal trials

DNA fingerprinting is an unreliable method for identifying suspects in criminal trials, according to leading molecular biologists. For practical and theoretical reasons, the fingerprinting method cannot provide conclusive proof that a person has committed a crime. Many are so skeptical about the reliability of the technique that they would not allow their DNA samples to be used in fingerprinting even if they were innocent in a criminal case. The scientists claim that the problem lies in the tendency of the DNA fingerprints to shift or stretch, almost like a pattern printed on rubber. However, even if the shifts did not occur, the patterns of DNA samples are almost impossible to compare and there is no basis for determining the probability that DNA fingerprints from two people could match by accident.

The FBI started to analyse DNA samples in 1989 and offers the service to all US police departments. DNA evidence has been presented in hundreds of criminal cases to date. The problems with DNA fingerprinting are more fundamental than the way that particular laboratories perform the analyses. (Source: New York Times, 29 January 1990)

How do proteins assemble in the cell?*

by Dr. Nicholas Price, Department of Biological and Molecular Sciences, University of Stirling

Proteins are the most important class of large molecules found in nature. A knowledge of the way their long chains assemble and fold within living cells, to bring certain parts of the chain close to one another, is not only a matter of fundamental

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* This article first appeared in Spectrum, No. 221, 1990/5.

biological importance. A better understanding and control of the process has many practical applications and is vital to the economics of producing proteins by genetic engineering techniques. Work supported by the UK Science and Engineering Research Council is steadily going forward to discover the many factors involved and the parts they play in this most complex process.

Proteins are made up of amino acids linked by what are known as peptide bonds to form polypeptide chains. There are 20 different amino acids, permitting a huge number of possible structures; for a chain 100 amino acids long there are 20^{100} (or approximately 1.3×10^{130}) possible combinations. Proteins that are biologically interesting range from about 50 to several thousand amino acids in a single chain.

The huge variety of protein structures is related to the variety of tasks they are intended to do in nature. Some proteins, known as structural proteins, have fairly regular structures, which can generate the mechanical strength important, for example, in beaks or nails, or in transmitting force, as tendons do. Others, termed functional proteins, have irregular, compact, globular structures and are meant to interact specifically with other molecules. Examples of the latter class include enzymes, which are natural catalysts; defence molecules, known as immunoglobulins, and carrier molecules such as haemoglobin, which carries oxygen in the blood.

Levels of structure

The structure of a protein can be defined at a number of levels, as shown in the first illustration (see page 27). Primary structure refers to the sequence of amino acids in the polypeptide chain. Secondary structure refers to the way in which the polypeptide chain can form localized elements of regular, three-dimensional structure such as helices or sheets. Tertiary structure refers to the overall folding of the polypeptide chain which brings various parts of the chain close to each other even though they may be widely separated in the sequence. Finally, quaternary structure refers to the arrangement of polypeptide chains in a protein which contains more than one such chain (for example, haemoglobin has four chains). The three-dimensional structures of over 150 proteins have been found by X-ray crystallography, groups at Bristol, Cambridge, London and Oxford Universities playing important parts in this work.

Although the structures of a large number of globular proteins have been mapped, the mechanisms by which the structures are acquired are much less well understood. In essence, the sequence of amino acids in a protein is fixed by the sequence of bases in the corresponding part of the organism's genetic store, known as deoxyribonucleic acid or DNA for short. The information in the base sequence of DNA is converted to information in the base sequence of a messenger called ribonucleic acid, or RNA, in a process known as transcription. The RNA sequence information is then translated into protein sequence information. At the ribosomes, the sites of protein synthesis, suitably activated amino acids are brought in the correct order to be joined together to form the polypeptide chain starting at the N-terminus. In some cases the polypeptide chain undergoes subsequent modification; so sugar groups can be added to give glycoproteins, or the chain can be split by the action of proteolytic enzymes. But in all cases, it is necessary for the polypeptide chain to fold up so as to form its characteristic three-dimensional structure. If the protein has more than one polypeptide chain the chains must also associate correctly.

Many proteins that are synthesized in one part of a cell perform their biological function in another part or may even be secreted from the cell. The movement of the protein product is called translocation; during this process the polypeptide chain may be modified. In eukaryotic cells, which are complex cells that have a distinct nucleus, the main store of genetic information is the DNA in the nucleus, seen in the second illustration (see page 28). Transcription of this DNA takes place within the nucleus and translation happens in the cytoplasm. However, there is also a small amount of DNA in mitochondria, which are responsible for producing the energy currency of the cell, adenosine 5'-triphosphate (ATP) and, in plant cells, in chloroplasts, the sites of photosynthesis. Many of the features of the genetic apparatus in these latter organelles resemble those of bacteria, where the cells, called prokaryotes, lack a distinct nucleus. This fact has led biologists to speculate that such structures represent an evolutionary remnant of free living prokaryotic organisms which then come together to form the more complex eukaryotic cells.

The DNA in mitochondria and chloroplasts has the capacity to code for only a small fraction of the proteins they need to carry out their work; most proteins are coded for by nuclear DNA and imported after synthesis in the cytoplasm. Some proteins are made up of polypeptide chains from different sources, one example being the chloroplast enzyme ribulose bisphosphate carboxylase (RubisCO) which catalyses the first step in the dark reactions of photosynthesis, the incorporation of carbon dioxide into the 5-carbon sugar ribulose bisphosphate in the absence of light. This enzyme consists of two types of polypeptide chain, each present in eight copies. The large chain, known as the L chain, is coded for and synthesized within the chloroplast, whereas the small or S chain is coded for by nuclear DNA and imported. I shall discuss the assembly of this complex enzyme later.

Folding of polypeptide chains

In an article in Spectrum 200, I set out some of the current ideas relating to the mechanism of folding of polypeptide chains. In practical terms it is extremely difficult to study the folding process during synthesis of proteins in the cell, so the more convenient experimental approach usually adopted is to study the refolding of proteins. A protein is denatured, which means it is made to lose its characteristic three-dimensional structure, by incubation with compounds such as guanidinium chloride or urea; this has no effect on the primary structure of the chain. At a known time, the denaturing agent is removed, usually by dilution, and refolding is monitored by the regain of biological activity or the re-appearance of the original structure. X-ray crystallography can be used only to determine structure in the crystalline state; in solution, techniques such as fluorescence and circular dichroism can be used to give structural information that is less complete but still valuable. The research groups of Dr. Creighton at Cambridge and Professor Pain at Newcastle-upon-Tyne have been very active in this type of study and, through these investigations, a general picture of the sequence of events in protein folding has begun to emerge (see third illustration - page 29). The first stage is the formation of elements of secondary structure such as helices,

sheets and so forth, a process dominated by the formation of weak interactions known as hydrogen bonds. In the next stage the polypeptide chain adopts a globular structure: the driving force for this process is the burying of hydrophobic or non-polar amino acid side chains away from water. This structure has been termed a molten globule by some workers, to emphasize the point that the amino acid side chains have much greater mobility than in the final structure. In the third and final phase the molten globule undergoes various structural adjustments to generate the final structure; the biological activity of the protein is usually regained in this final phase.

Association of chains

A research group led by Professor Jaenicke in Regensburg, Federal Republic of Germany, has made a detailed study of the processes of refolding and re-assembly of several proteins with multiple polypeptide chains. One conclusion from the work is that association of the individual polypeptide chain is highly specific. For example, there is no evidence for the formation of hybrid species when pairs of such proteins are allowed to refold and re-assemble after denaturation (the conditions used for denaturation also lead to dissociation of the protein into individual polypeptide chains as well as the unfolding of each chain). The overall process consists of a sequence of folding and association steps; biological activity is sometimes shown by the intermediate, isolated, folded polypeptide chains before association occurs. In any case, however, the association must occur when the individual polypeptide chains have folded enough to generate a specific recognition site and thereby allow the interaction with another chain to take place.

A good model?

The question remains as to how good a model the refolding of denatured proteins is for the process of folding during the synthesis of proteins in the cell. Some evidence suggests that it may be a reasonable model, and that a number of proteins consisting of a single polypeptide chain are able to refold at a rate commensurate with the likely rate of folding *in vivo*, which is estimated to be complete within 10 seconds. In addition the recovery of biological activity on refolding is close to 100 per cent in several cases. However, in other respects it has become clear that refolding is not a perfect model, and this seems to be the case especially with larger and more complex proteins. In these, the rate of refolding is not as rapid as folding *in vivo* and the yield of active protein is often much less than 100 per cent. The main side reaction appears to be the formation of large aggregates, probably through non-specific associations between the exposed non-polar, or hydrophobic portions of individual folded units or polypeptide chains during the refolding process (see third illustration).

There is no strong evidence that such large aggregates form during the synthesis of proteins in the cell, so it is necessary to consider how this type of side reaction could be avoided. One possibility is that during the process of translation the growing polypeptide chain begins to acquire three-dimensional structure, a process termed co-translational folding. From studies with model polypeptides it has been found that stable structures can be formed by chains containing 30 or fewer amino acids, and the rate of acquisition of such structures is much faster than the rate at which the polypeptide chain grows (estimated at between five and 10 amino acids added per second). Co-translational folding could therefore allow the growing polypeptide chain to form one or more folded units in a defined sequence from the N-terminus which would then discourage incorrect associations leading to the formation of aggregates. This is to be contrasted with the refolding type of experiment, in which the entire polypeptide chain is allowed to fold 'at one time' with no specified sequence of events from one end of the chain. It is not difficult to envisage that if this is so, incorrect folding and association could happen.

The rate of formation of correctly folded protein *in vivo* can be accelerated by the modifications occurring after translation. Secreted proteins often contain disulphide bonds in which pairs of cysteine side chains are linked. Formation of the correct disulphide bonds during refolding is often very slow; however, there is an enzyme known as protein disulphide isomerase which speeds up the process considerably. Professor Freedman and his colleagues at the University of Kent, Canterbury, have helped to characterize the role of this enzyme in detail. Other recent results show that the addition of sugar units to the polypeptide chain, to give glycoproteins, may help to discourage incorrect aggregates forming during the folding of proteins.

Studies of protein folding and refolding have important practical applications as well as being of fundamental biological interest. Advances in recombinant DNA technology have allowed the genes coding for a number of eukaryotic proteins to be expressed in prokaryotic systems. For example in work done at Celltech, at Slough, southern England, the gene that codes for prochymosin, the precursor of chymosin, otherwise known as rennin, has been cloned and expressed in the bacterium *Escherichia coli*. Rennin is an enzyme normally obtained from calf stomach; it is used to curdle milk in the process of making cheese. Producing prochymosin by this strain of genetically engineered bacteria is obviously much more convenient than isolating the enzyme from calf stomach. Unfortunately, a number of proteins, including prochymosin, when expressed in *E. coli*, form an insoluble aggregate within the bacterial cell. Recovering the desired protein(s) involves solubilizing the cell contents, usually with the denaturing agent guanidinium chloride, and then allowing the protein(s) to refold when the denaturant is removed. This refolding step is often the most difficult and least understood part of the whole process. Obviously, better control of it is vital to the economics of protein production by recombinant DNA technology.

Translocation of proteins

Because many proteins are translocated from their site of synthesis to destinations either inside or outside the cell, several questions arise about this process. First, how is a protein directed to its particular destination? Second, in what form is the protein during translocation? and third, how is the protein assembled to its mature form? Work over the last five years or so has helped to provide some answers to these questions.

First, the direction of proteins to a target appears to be a result of distinct types of sequences of amino acids at the N-terminal end of polypeptide chains, the first part to be synthesized. For proteins which are destined for secretion from the cell, the sequence is generally 15-35 amino acids long and, apart from a basic amino

acid at or near the N-terminus, there is a high preponderance of hydrophobic amino acids. Studies in the USA have shown that this "signal" sequence directs a ribosome to the surface of the endoplasmic reticulum membrane (see the second illustration) and then helps to thread the growing polypeptide chain across this membrane into the internal space, or lumen. The signal sequence is removed by an enzyme within the lumen and the polypeptide chain is usually modified, for example by formation of disulphide bonds or the addition of sugar residues, prior to transport to the Golgi apparatus and subsequent secretion from the cell.

Proteins which are destined for import into the mitochondrion also contain the appropriate targetting information in their N-terminal sequences; there are different types of sequence for the different destinations within the mitochondrion (see second illustration). For example, the typical targetting sequence for import into the matrix of the mitochondrion contains a regular arrangement of basic amino acids with very few carboxylic acid groups. Once the protein has arrived in the matrix the sequence is removed by the action of one or, in some cases two, proteolytic enzymes. Variations of the matrix-targetting sequence allow the other possible destinations within the mitochondrion to be specified. Similar targetting sequences have been found for proteins that are to be imported into chloroplasts in plant cells. How important these sequences are has been demonstrated by a number of elegant experiments involving recombinant DNA technology in which so-called hybrid polypeptide chains have been constructed. The hybrid chains contain a targetting sequence added on to the N-terminal end of the polypeptide chain of a protein which would not normally undergo translocation. The hybrid protein undergoes the translocation specified by the target sequence so that a protein can be directed to a destination different from its normal one.

Relating to the second question, several lines of evidence point to the fact that the protein is in a largely unfolded state during its translocation, which means the polypeptide chain can be envisaged as being threaded through the appropriate membrane rather than being transported as a compact globular unit. So the C-terminal portion of one of the polypeptide chains of the enzyme involved in synthesizing ATP in the matrix of the mitochondrion can be digested by an externally added enzyme at the same time as the N-terminal targetting sequence is being removed by the appropriate enzymes in the matrix. This clearly shows that the polypeptide chain has an extended unfolded structure, traversing both the inner and outer membranes of the mitochondrion.

With regard to the third question, the transition of the translocated protein into its final, mature form is the least understood part of the process. In view of the finding that the protein is translocated in a largely unfolded state, it is particularly puzzling that certain mature mitochondrial proteins cannot successfully refold after denaturation. We have shown that glutamate dehydrogenase, an enzyme involved in the metabolism of amino acids, and another enzyme called citrate synthase which plays a part in the tricarboxylic acid or TCA cycle, are mitochondrial proteins of this type. Other workers, including Professor Ellis at Warwick University, have reported that the chloroplast enzyme RubisCO cannot be re-assembled after denaturation.

Clues as to how the assembly process might occur <u>in vivo</u> have recently begun to emerge. In the case of RubisCO, Professor Ellis and his group have found that there is a binding protein within the chloroplast which forms a tight complex with the large (L) polypeptide chain of the enzyme; (the chain is made within the chloroplast). The complex is known to be dissociated when ATP is added. Isolated L chains cannot refold after denaturation, but form large aggregates instead. It is proposed that, in the chloroplast, L chains are released from the complex formed with the binding protein in a controlled way to allow the correct associations with the imported small (S) chains to occur (as in the fourth diagram: see page 29). Analogous binding proteins have now been reported in other biological locations.

Illustration 1

The levels of protein structure: (a) A typical amino acid. The various amino acids differ in the nature of the side chain, R, which may be basic (positively charged), acidic (negatively charged) or neutral (uncharged). If R is a hydrocarbon, the side chain is described as non-planar or hydrophobic and the amino acid's solubility in water is reduced. (b) A dipeptide in which two amino acids are linked by a peptide bond (boxed). (c) The right handed helix which represents an element of secondary structure. For clarity the side chains have been omitted. Broken lines represent the hydrogen bonds which help to stabilise the structure. (d) The tertiary structure of one of the polypeptide chains in haemoglobin. There are eight main helical segments in the chain. Oxygen binds to the iron atom in the centre of the haem group. (e) The quaternary structure of haemoglobin, in which four polypeptide chains are associated.

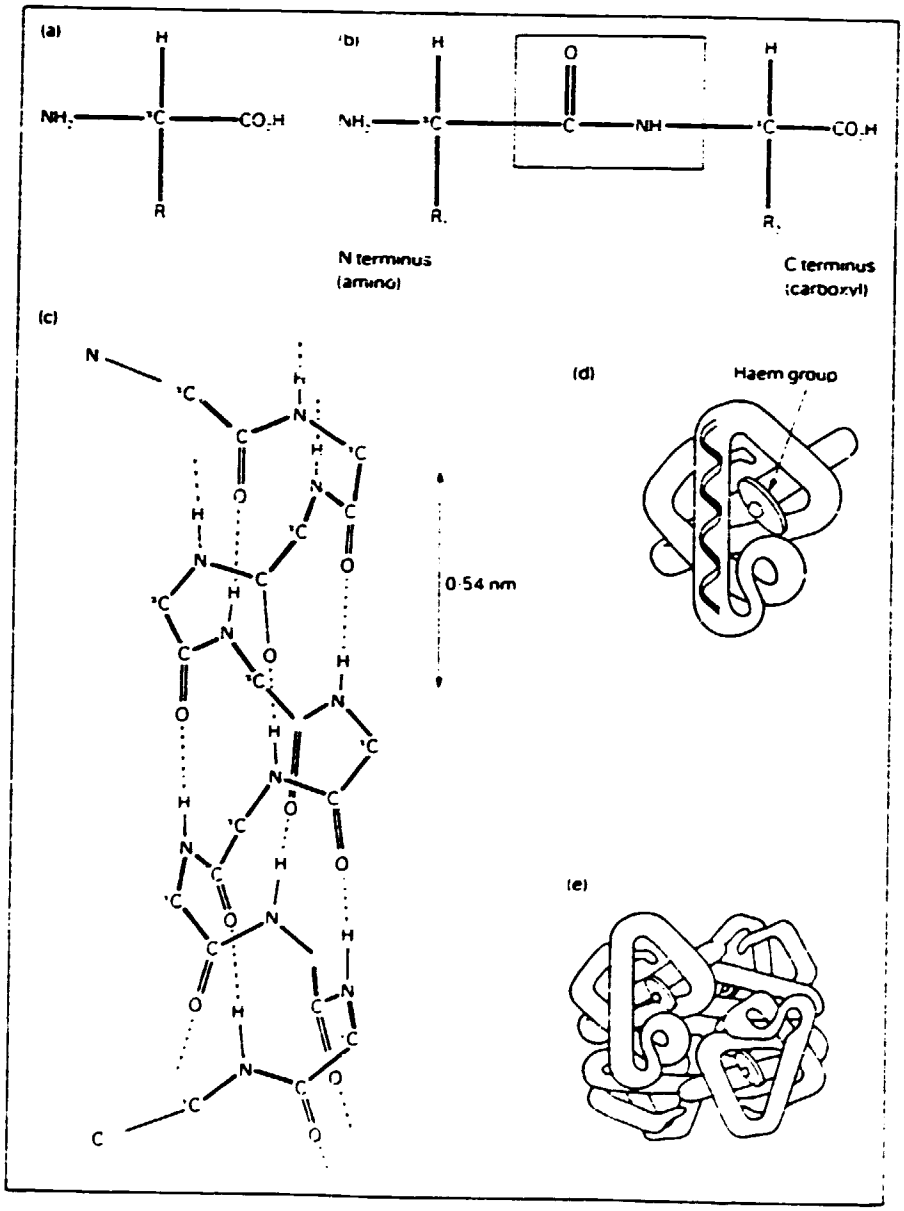


Illustration 2

The structure of a typical animal cell as seen with the electron microscope. For simplicity only some of the rough endoplasmic reticulum (with ribosomes bound) is shown. Similarly, only some of the free ribosomes are shown. (Inset) The structure of the mitochondrion, showing its different compartments.

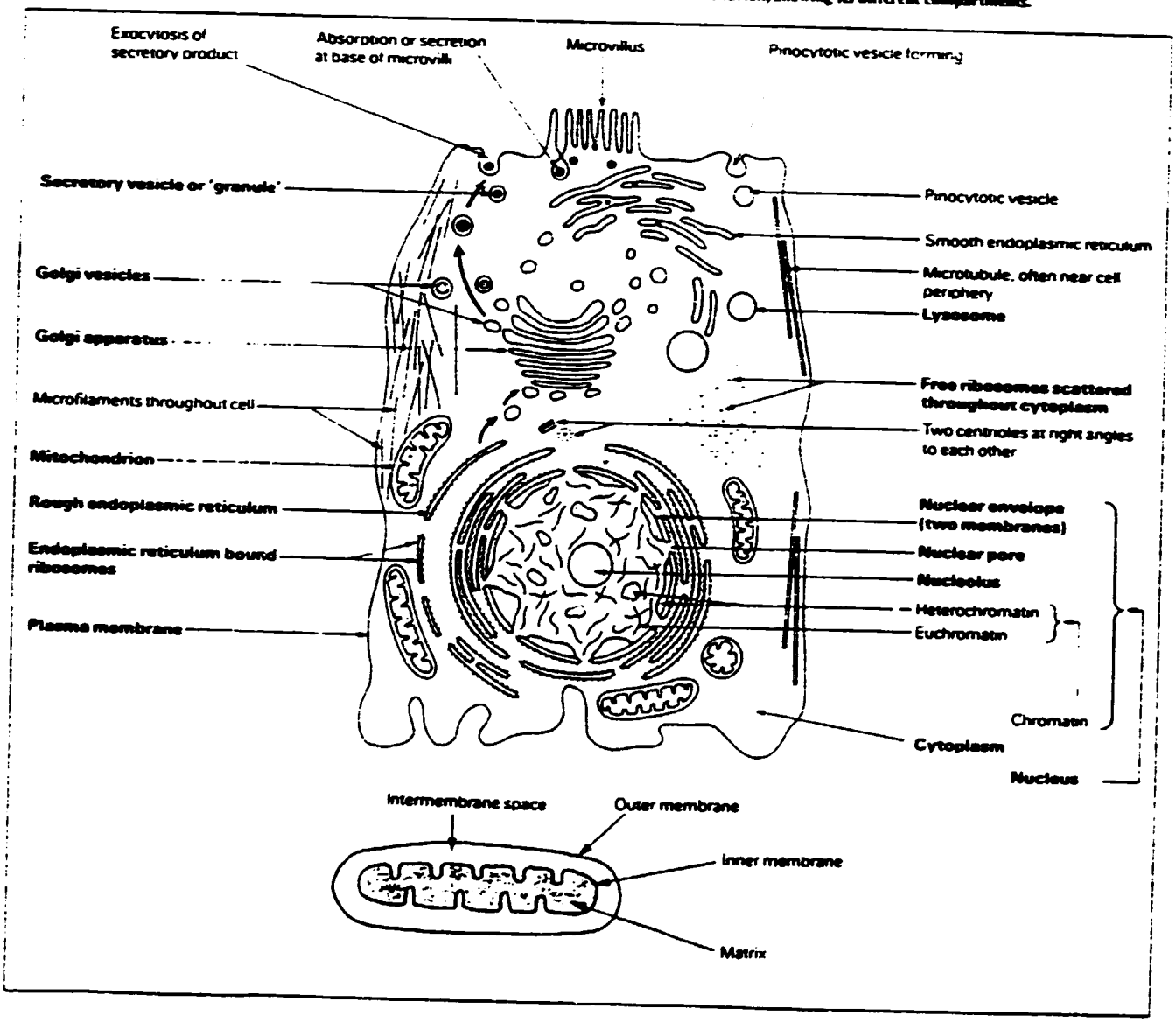


Illustration 3

A model for the folding of proteins. In the first stage, elements of secondary structure (helices and sheets, shown as ribbons and arrows respectively) are formed rapidly. These act as centres for growth of regular structure and formation of a globular structure (stage two). In stage three the polypeptide chain undergoes a number of structural adjustments to generate the final structure. Formation of aggregates could result from incorrect associations of parts of the intermediates formed in the first two stages.

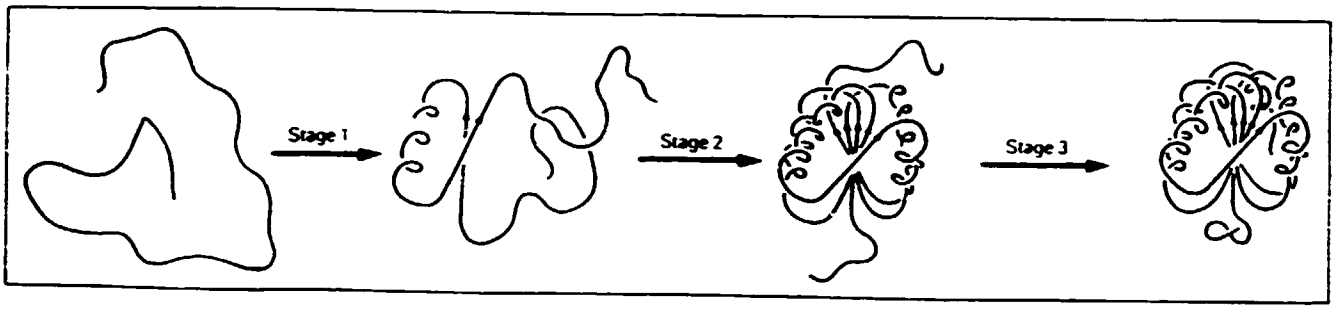
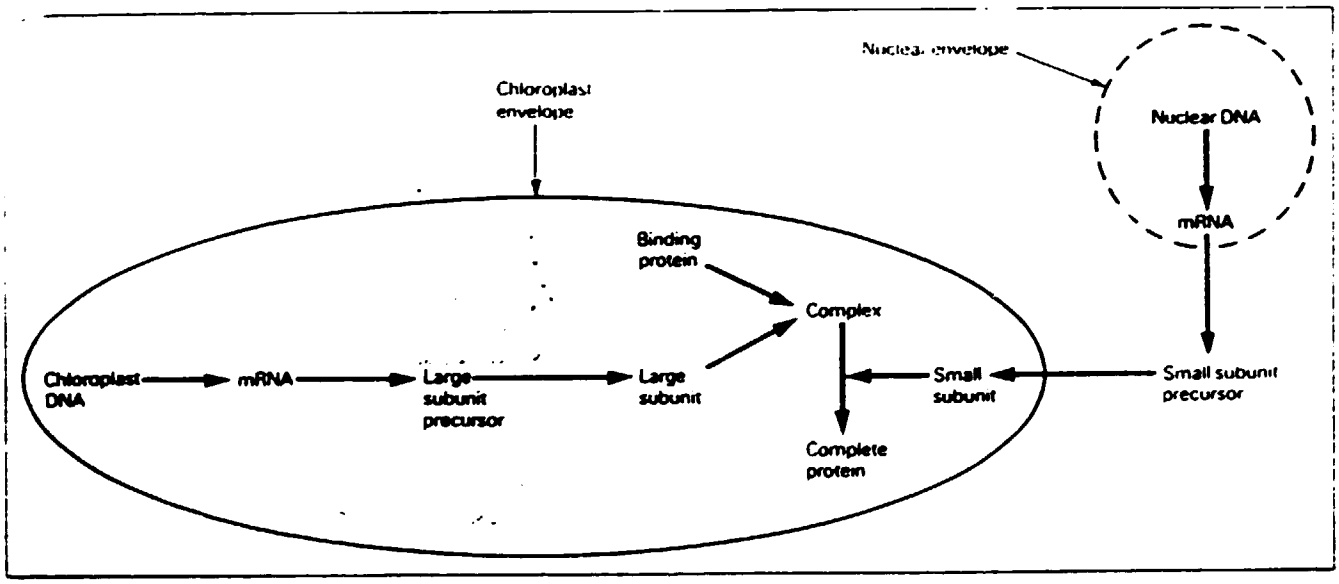


Illustration 4



The assembly of RubisCO in the chloroplast, showing the formation of the large and small subunits. For simplicity, the fact that the binding protein is coded for by nuclear DNA and imported into the chloroplast is not shown.

D. APPLICATIONS

Medical and pharmaceutical applications

Mabs against tetanus

Researchers at the Morinaga Institute of Biological Science (Yokohama City, Kanagawa Prefecture) have developed human monoclonal antibodies for tetanus. The antibody is available in six types and can be mass produced for clinical use. It neutralizes tetanus toxin at better than 0.001 IU/109 microgram efficacy or 10-100 times the efficacy of conventional globulin tetanus antitoxins. In addition, because the monoclonal antibody is not manufactured directly from blood like globulin antitoxins, there is no associated threat of viral infection. (Extracted from New Technology Japan, January 1990)

Arthritis drug in UK trials

Bio-Technology General Corporation has initiated a clinical trial aimed at testing the safety and efficacy of its recombinant human superoxide dismutase (SOD) in rheumatoid arthritis patients.

The study is being co-ordinated at the London Hospital in the UK. The company says the responsible clinicians have been at the forefront of clinical research efforts attempting to assess the causal relationship between the presence of oxygen free-radicals and the development of arthritis in the joints.

The group has demonstrated increased levels of oxidation products as well as elevated amounts of a superoxide-generating enzyme, xanthine oxidase. In addition, the group has shown that the arthritic joint generates damaging free-radicals during a rest-exercise cycle, characteristic of reperfusion injury.

It is felt treatment of rheumatoid joint inflammation with injections of the free-radical scavenger SOD directly into the joints may prove to be clinically valuable by blocking the damaging effects of free-radicals.

Although individuals with osteoarthritis have been treated wi h a non-human SOD (bovine) for more than a decade in Germany, BTG says it believes its clinical trial to be the first ever to evaluate recombinant human SOD in the treatment of rheumatoid arthritis.

Meanwhile Synergen has discovered a protein that can block the effects of rheumatoid arthritis. The protein inhibits interleukin-1, which can cause the pain and swelling and cartilage decay of rheumatoid arthritis. Interleukin-1 is normally involved in fighting infections. The newly discovered protein apparently blocks interleukin-1 receptors on cells. Synergen says the protein will not reverse damage already done by rheumatoid arthritis, but it will halt progression of the disorder. Testing on humans could begin by end-1990. Synergen and Hoffmann-La Roche will jointly develop a synthetic equivalent of the protein.

The new drug might be valuable in treating a variety of immune system disorders, such as diabetes, asthma, lupus, etc. (Source: Chemical Marketing Reporter, 19 March 1990 and New York Times, 30 January 1990)

Drug found to treat osteoporosis

A drug sold by Proctor & Gamble's Norwich Eaton pharmaceuticals subsidiary reverses the bone damage from osteoporosis, a disease suffered by a quarter of elderly women, suggests a study published in the New England Journal of Medicine.

The authors of the paper, from Sundby Hospital in Copenhagen conclude that Didronel (disodium etidronide) has a significant effect in preventing fractures of the degenerative disease. The work, based on a three-year trial of 66 women suffering the bone embrittling disease, found patients taking the drug averaged a 5 per cent gain in bone mass while those on the placebo showed a 3 per cent loss.

Etidronide is currently used to treat Paget's disease of the bone. However, the drug is believed to modify the dynamic equilibrium that exists of mineral elements (such as calcium) in the bone. Minerals are continuously absorbed from and deposited in bone tissue, with the time for a complete turnover about three months. The scientists believe Didronel partially inhibits absorption.

The study provides the first conclusive evidence that drugs can increase bone mass and prevent fractures in women affected by the disease. Current therapies for treating osteoporosis, such as hormone replacements and fluorides, tend to slow rather than reverse the deterioration. Both existing treatments have some disadvantages, for example, questions over the integrity of the new bone. However, HRT does have some beneficial secondary effects.

Norwich Eaton hopes the drug will be shown to complement existing therapies and studies may show synergy. (Source: European Chemical News, 14 May 1990)

Mabs-drug reduces septic shock deaths

Centocor's human monoclonal antibody drug Centoxin significantly reduces mortality in patients suffering potentially-fatal gram-negative bacteraemia and sepsis, the company's scientists intend to report.

An abstract of a report, which will be presented to the American Society for Clinical Investigation, claims a 39 per cent drop in mortality rate compared to a placebo. Moreover, in septic shock, a more serious condition, the mortality drops by 42 per cent.

Last year, the rival US biotech company Xoma published trial results of its Mabs-based drug Xomen-E5. Xoma reported a similar reduction in mortality for sepsis, but published no results to compare efficacy in septic shock.

Both drugs await approval in the US and Europe and the first launches are expected this year. The two drugs could be the first big selling Mabs-based pharmaceuticals. (Source: European Chemical News, 30 April 1990)

US team describes oral insulin administration

An oral form of the protein insulin may soon replace subcutaneous injections for diabetic patients. Scientists at the Medical College of Ohio have developed a way of overcoming the

problem that insulin taken orally is broken down by the body's digestive enzymes in the same way as other proteins in food.

Professor Murray Saffran and colleagues have coated insulin in gelatin capsules with a waterproof plastic, which appears to protect the capsule from destruction in the stomach and small intestine. When the capsule reaches the large intestine (colon), naturally occurring bacteria break the plastic coating, allowing the insulin to be released and absorbed.

Saffran described his work at the recent Biochemical Society meeting at Bath, UK, where he explained that studies in diabetic dogs (made so by the removal of the pancreas) had shown the oral formulation to control blood sugar levels.

Because the capsule takes some time to travel through the alimentary canal, Saffran explained that it would be taken about four hours before meals, in anticipation of raised glucose levels. He believes it will be suitable for insulin dependent diabetics.

Saffran said that, although insulin administered through the colon has a low bio-availability, the proximity of the colon's blood vessels to the liver makes it sufficient to control diabetes. His team has studied the use of an adjunct (5-methoxysalicyclic acid), a derivative of aspirin, to increase absorption.

In contrast, insulin delivered subcutaneously enters the general blood circulation and only a small part reaches the liver. Apart from the advantage of convenience, the oral form may reduce complications that occur from insulin in the blood stream acting on muscles, causing hypertension, heart disease and kidney failure.

The oral formulation is different to the technique used by Cortecs, a London-based drug delivery firm, but the approaches lead to the same effect. Cortecs uses a microemulsion of insulin in fatty acid and fat molecules to protect the insulin. (Source: European Chemical News, 23 April 1990)

IL-2 products in Europe

Cetus and Hoffmann-La Roche have agreed to co-market their interleukin-2 (IL-2) products in Europe. Under the agreement, each will market both Proleukin and Roferon-A, respectively Cetus' and Roche's products, in Switzerland and all EC countries, with the exception of Denmark and Greece. Proleukin is approved for renal cell carcinoma, and Roferon-A approved for various cancers and viral infections in most of Europe. The two companies are collaborating on the development of IL-2 for additional indications. (Source: European Chemical News, 30 April 1990)

TNF success

BASF's Knoll pharmaceutical subsidiary has reported success in recent clinical trials of tumour necrosis factor (TNF). Knoll claims that patients suffering from kidney cancer have shown a "substantially" improved recovery rate when treated with TNF combined with human immune system proteins, such as alpha 2 interferon. This combination has resulted in tumour recession of 50-100 per cent in trials conducted in Hamburg. Hearings on BASF's application to build a plant to manufacture TNF at Ludwigshafen are scheduled to restart soon. (Source: European Chemical News, 4 April 1990)

Genex offers "research" wares

Genex Corp., Gaithersburg, Md., sold its first three bottles of Adhera-Cell™, a recombinant bio-adhesive based on the "glue" that anchors blue mussels to seashore rocks. The protein, which differs 60 per cent from the natural amino-acid sequence, is used by laboratories as a surface support for cultured, anchorage-dependent cells. Although it has not yet started clinical trials with this glue, Genex plans also to develop the engineered protein as a dental and ophthalmic adhesive, a surgical suture replacement and for use in skin grafts.

Two other products are also being developed for medical applications: Anti-fluorescein SCA™, a single-chain, protein-engineered, antigen-binding molecule that contains only the non-antigenic Fv portion of the antibody, is designed for cytochemistry studies. Concurrently, Genex is in Phase I trials with the recombinant molecule linked to a radioactive marker, as an imaging agent for colorectal cancer. GammaBind Fab Separator, which isolates IgG antibody fragments in research and scale-up settings by binding only to the Fc region of the target molecules, is being readied as a therapeutic. Genex says the cloned protein will be useful in aphoresis columns to purify the blood of patients with autoimmune diseases. (Source: McGraw-Hill's Biotechnology Newswatch, 19 March 1990)

First human gene-therapy trial gets first of seven federal permissions

Based on the success of recent gene-transfer experiments, the Institutional Biosafety Committee of the National Institutes of Health (NIH) has approved the first gene-therapy experiment involving humans. If the protocol passes several other governmental reviews, clinical trials in patients who lack the functional gene for the enzyme adenosine deaminase (ADA) could begin before the end of this year.

Under the proposal by W. French Anderson of the National Heart, Lung and Blood Institute (NHLBI) and R. Michael Blaese of the National Cancer Institute (NCI), the ADA gene sequence will be inserted *in vitro* into T cells removed from patients with severe combined immunodeficiency (SCID), a genetic disease caused by the body's inability to produce ADA. The transformed T lymphocytes, grown in large numbers in the laboratory, will then be injected back into the patient's bloodstream to produce the missing enzyme.

Anderson's team has already accomplished the first step - successfully inserting the gene *in vitro* into the T cells of SCID patients, so they would produce ADA. A major goal of the clinical trials will be to learn how long the cells continue producing the missing enzyme, after being injected into the patients.

Anderson had teamed up with Blaese and Steven Rosenberg of NCI to design a protocol for inserting a bacterial neomycin-resistance gene into tumour-infiltrating lymphocytes (TIL cells) administered to terminal cancer patients. This "gene-transfer" experiment allowed Rosenberg to track the TIL cells during the patient's treatment, but otherwise contributed nothing to the transformed cells' therapeutic effect. However, before it could be conducted, NIH's institutional biosafety committee had to be convinced that patients would suffer no adverse effects from the retrovirus used to carry the resistance-factor marker gene into the TIL cells, Anderson notes.

The eventual safety and success of the still-ongoing TIL experiments laid the groundwork needed to proceed with the side-tracked ADA gene-therapy trials. Anderson's new protocol duplicates as closely as possible the TIL-gene study design. The same mouse retrovirus will be used to transform the same kinds of T cells, except that the DNA sequence actually inserted in vivo will be a human gene that expresses ADA, rather than a bacterial gene for neomycin resistance.

If the ADA gene-therapy experiment with T cells succeeds, Anderson says he will finally return to his original concept - transforming bone-marrow cells to produce normal lymphocytes over the patient's lifetime - thus, a permanent cure for SCID. (Source: McGraw-Hill's Biotechnology Newswatch, 19 March 1990)

Cancer testing kits

Cancer research has a long road ahead in developing "cancer-testing kits". The importance of genes as being a factor in cancer development, and other genes that suppress cancer, prompted the thought that genes could be a help in identifying people with the highest risk of getting the disease. Testing kits, however, are slow in developing, partly due to problems in identifying the right gene. While about 50 genes that can cause cancer have been identified, just 12 have been linked to specific tumours that arise in humans. (Extracted from The Economist, 23 February 1990)

Heart drug debate grows

Two new studies are adding to the debate over which drug should be used to reduce deaths from heart attacks. Three products - streptokinase, TPA and "Eminase" - are all vying to supply this critical market.

The latest is a 180-patient trial comparing SmithKline Beecham's "Eminase" (anistreplase) with Genentech Inc.'s "Activase" brand TPA. French researchers reported at the American College of Cardiology that the two were basically matched in their ability to open blocked arteries and maintain the pumping function of the heart.

According to SKB, the only difference found between the two agents in the study was in the fibrinogen level (clotting factor): it was much lower in "Eminase" patients at six hours than in TPA patients, and is attributed to the longer half-life of "Eminase".

Genentech, however, considers this finding a plus for TPA, because bleeding complications can be more easily halted when the drug clears from the body more quickly.

SKB also points to ease of injection as a plus for "Eminase", because delays in the hospital can be reduced by giving the drug in an emergency room or before the patient reaches the hospital. "Eminase" requires a two-to-five minute injection while streptokinase needs a 30-to-60 minute infusion and TPA a three-hour infusion.

Earlier, a much larger study found that streptokinase, marketed by Hoechst-Roussel Pharmaceuticals Inc. as "Streptase" and by Kabi as "Kabikinase", was basically equal to TPA in preventing mortality.

However, this study is disputed as well, specifically regarding when a supporting injection of the anti-coagulant heparin should be injected. In the study, heparin was not given until 12 hours after patients reached the hospital. Genentech contends the study is flawed because earlier injection is called for.

All three heart agents have yet to be compared in a head-to-head study, although the ISIS-3 study now under way will attempt this with more than 40,000 patients. For now, the most notable difference between the three is in price: TPA is about $2,200 per dose, "Eminase" is $1,700 and streptokinase is around $200.

Meanwhile, another new study published last week in the New England Journal of Medicine indicates a daily dose of aspirin can cut in half the risk of strokes in people who suffer from an irregular heartbeat.

The study of 1,244 adults found that aspirin cut the risk of stroke by 50 to 80 per cent among patients suffering from atrial fibrillation, a common heart condition that leads to about 70,000 strokes every year. The research also discovered a similar protection against stroke among patients taking warfarin, a prescription anticlotting drug. (Source: Chemical Marketing Reporter, 26 March 1990)

Will monoclonals out-do lasers in mopping up remnant cataract cells?

If Dominic Man-Kit Lam's toxin-linked monoclonal antibodies perform as well in vivo as they do in vitro, they will offer eye surgeons an alternative to lasers in correcting secondary cataracts. Acting as molecular scalpels, Lam's monoclonals - specific to human-lens epithelial cells, and coupled to cell-killing ricin A - will mop up and wipe out the opaque monolayer of cells that often grows back to cloud vision after surgical removal of the primary cataract.

Lam, who is founder and chairman of Houston Biotechnology, Inc., calculates that with half of the million cataract patients requiring secondary cell scavenging, at $1,000 each, his antibody system would confront a half-billion-dollar market. At $100, it "would be cost-effective, and hopefully viable commercially".

Cataract extraction is one of the most popular surgical procedures in the world, with more than one million done in the USA alone each year, at a cost of $2,000 to $3,000. In half of these cases, the few cells that the surgeon's instruments cannot reach creep back to cover the interior rear wall of the lens capsule. At this point, Lam explained, current procedure "is to blast a hole through the wall with a laser". This costs, typically, another $1,000 and risks damaging the retina or other parts of the eyeball behind the lens.

If the antibody-toxin conjugate is instilled at the time of the primary surgery, it eliminates the need for a return engagement, by extirpating every last opaque epithelial-cell remnant. Because this is a one-shot procedure, the murine component of the monoclonal would not risk immune reaction or rejection.

In many instances, the ophthalmologist inserts a plastic lens into the lens cavity after removing

the primary cataract. This already approved medical device can be coated with the conjugate, as a more elegant delivery approach than instillation.

Still in the preclinical testing stage, the antibody-ricin conjugate has "proven efficacious in selectively destroying lens epithelial cells". (Source: McGraw-Hill's Biotechnology Newswatch, 16 April 1990)

Triton Biosciences releases two new cancer research products

Two new monoclonal antibodies are now available, for research use only, in detecting, diagnosing and monitoring the course of certain cancers, it was announced by the Diagnostics Division of Triton Biosciences Inc.

The first, RB Gene Product Antibody (Mab1), is for use in studying the role of the retinoblastoma (RB) gene in the pathogenesis of cancer, whilst the second, a monoclonal antibody against HPV-18 E7, is for studying the role of human papillomavirus (HPV) in malignant transformation.

The RB gene was identified as the first "tumour suppressor" gene in the childhood cancer, retinoblastoma. Retinoblastoma occurs during the first four years of life, affecting about one in 20,000 live births world-wide.

Tumour suppressor genes are thought to function by suppressing the cell's ability to proliferate. When this regulation is removed through genetic loss or mutacion of the gene, the cell is then able to proliferate uncontrollably, one of the characteristics of cancer. The loss or inactivation of the RB gene was established as the key event in retinoblastoma tumour development.

RB gene mutations have also been demonstrated in osteosarcoma, small cell lung carcinoma, breast cancer, bladder carcinoma and prostate carcinoma cell lines or human tumours. Triton researchers are exploring possible applications of the RB Gene Product Antibody (Mab1) in these other indications.

The RB Gene Product Antibody (Mab1) has been shown to detect, by radioimmunoprecipitation and Western blot analyses, an unphosphorylated protein of M_r 110 kD. An associated, less distinct, variable region of M_r 100 kD to 16 kD was also identified, which represents the various phosphorylated forms of the RB protein.

Triton researchers established the specificity of the antibody by positive reactivity with bladder carcinoma and normal foetal fibroblast cell lines known to express the RB protein. Both Western blot and radioimmunoprecipitation have been blocked by antibody readsorption with a synthetic peptide from the published RB DNA sequence. Negative reactivity was demonstrated with a retinoblastoma cell line known to lack the RB gene.

Specific types of human papillomavirus (HPV) have been shown to be associated with cervical cancer. HPV types 16 and 18 viral DNA have been detected in up to 90 per cent of cervical carcinoma biopsies. Independent research has demonstrated that the HPV type 16 early protein, E7, has the capacity for cellular transformation, a function that may be of importance in the multistep progression of cervical cancer.

Triton's HPV-18 E7 monoclonal antibody has been shown to detect the HPV-18 E7 gene product in Western blot and immunoprecipitation analyses. Immunohistochemical staining performance on human tissues as well as correlation of staining performance with DNA probe techniques is under investigation.

The specificity of the antibody was established by positive reactivity with a bacterially-derived E7 fusion protein and the expressed E7 protein in human cervical carcinoma cell lines containing HPV-18 DNA. Negative reactivity was shown with other HPV fusion proteins and with other cerical carcinoma cell lines containing HPV-16 DNA or negative HPV DNA.

The HPV-18 E7 antibody joins Triton's related research product, a monoclonal antibody to HPV-16 E7, which was released in September 1989.

Triton Biosciences Inc. is a wholly-owned health care subsidiary of Houston-based Shell Oil Company. The company is focused on the development and commercialization of diagnostics and pharmaceuticals for cancer, viral diseases and other serious illnesses. (Source: Company News Release, May 1990)

AIDS vaccine gets FDA okay for use in US

The US Food and Drug Administration has approved nationwide testing of a post-exposure AIDS vaccine developed by Dr Jonas Salk, who 35 years ago pioneered the polio vaccine. Dr. Salk's vaccine will first be given to about 60 people who are infected with the AIDS virus, but who have not developed any symptoms of the disease.

FDA officials say the new vaccine manufactured by Immune Products Ltd. of San Diego, California, has already been tested in chimpanzees and about 100 AIDS-infected people in California, which has its own AIDS drug testing programme.

It is hoped the vaccine, which consists of inactivated or "killed" AIDS virus (HIV), will spur an immune response that will halt or even reverse the spread of the deadly virus in people suffering from AIDS. It can take 10 years or longer from the time people are infected with HIV until they develop AIDS.

The Salk vaccine is the third experimental AIDS vaccine FDA has approved for human testing. Sites of the FDA-sanctioned tests, which will run for nine months, were not named. Last June, Dr. Salk reported two HIV-infected chimps lost all signs of the AIDS virus after receiving his vaccine. Results of early tests in HIV-infected patients have not been made public. But researchers have asked California health officials for permission to widen testing to include 10 uninfected people at very low risk of AIDS infection.

Dr. Salk has pledged to be the first uninfected person to take the AIDS inoculation. The vaccine carries the potential for infection if all the virus used is not completely killed.

In theory, researchers say one of the most effective ways to prevent the spread of AIDS would be through vaccination. But because of the complex and rapidly mutating structure of the AIDS virus, most experts predict any vaccine is at least five years away.

Tests of a genetically-engineered vaccine made from an insect virus, produced by Microgenesys Inc. of West Haven, Conn., started in August 1987, while Bristol-Myers Squibb Company of New York received approval to test a genetically-engineered vaccine made from the vaccinia virus in November 1987. Test results reported so far have not been impressive.

Developers of a fourth vaccine, made by Alpha 1 Biomedicals Inc. of Washington, D.C., and Cel-Sci Corporation of Alexandria, Va., were granted permission by California officials to conduct human tests in that state earlier this month.

Meanwhile, FDA is asking Bristol-Myers Squibb Company for more information about an experimental AIDS drug following reports of six deaths from adverse reactions. The pharmaceutical company confirmed that drug-related inflammation of the pancreas killed five of 8,000 AIDS-infected people who received dideoxyinosine, or DDI, in a new "expanded access" plan, as well as one of 700 patients who got the drug through traditional tests. (Source: Chemical Marketing Reporter, 19 March 1990)

Major development in AIDS drugs

SmithKline Beecham (UK) reports a major development in a new kind of drug to treat AIDS victims. A similar development was reported by Upjohn. Scientists at both firms report discovering a number of compounds that make the AIDS virus impotent in test-tube experiments. The researchers say the chemical compounds are being used as prototypes for what they anticipate as a new category of drugs to prevent the HIV-1 (human immunodeficiency virus) from reproducing itself in cells that have not been infected. The two drug firms, as well as researchers at Merck, Abbot Laboratories, Monsanto and others, are trying to find a chemical that can thwart the work of an enzyme known as protease. (Extracted from Wall Street Journal, 26 January 1990)

Roche plans AIDS drug collaboration

Hoffmann-La Roche and Genetics Institute are to collaborate on research directed at finding therapeutic agents for treating AIDS.

The collaboration will focus on reverse transcriptase (RT) inhibitors, drugs which block a vital enzyme unique to the HIV and without which the virus cannot replicate.

The agreement binds the two companies to exchange technical information and contribute research materials, but fund their own programme. In addition, GI will have marketing rights to any protein or biosynthetic peptide and Roche will have rights to any synthetic molecule arising from the collaboration. Both companies will receive royalties from each other if a product is commercialized. (Source: European Chemical News, 26 March 1990)

French institute claims progress with AIDS vaccine

A potential vaccine against HIV has produced encouraging results at a laboratory in Paris. Two chimpanzees, each of which received different but related inoculations, appear to be free of the virus six months after French researchers deliberately infected them with it. But Marc Girard, the deputy director of the Pasteur Institute, who led the team, warns that many obstacles remain before the experiments can be called a success.

The search for a vaccine against HIV has so far eluded scientists, partly because the virus mutates rapidly and - unlike other human viruses - comes in many different forms that make the body produce many different types of antibodies. So even if scientists could design a vaccine that could stimulate the body to produce one or more of these antibody types in people, they might not protect completely against infection.

A second problem is that, once infected with HIV, the immune system's cells fail to respond normally. So the best hope, says Girard, is to produce a very high level of immunity.

The first chimp received three successive inoculations. First, Girard gave it genetically-engineered vaccinia virus - the basis of traditional smallpox vaccine - with an inserted gene from HIV. This gene makes the vaccinia produce the protein from HIV's coat, called gp160, which, in theory, should stimulate antibodies capable of "neutralizing", or engulfing, the virus. However, the chimp failed to make antibodies.

Next, the French team gave the chimp a direct inoculation of purified gp160. This time, the chimp produced some antibodies, but not the vital neutralizing ones. Finally, the researchers gave the chimp a synthetic peptide - the building block of protein - copied from a loop in the protein coat of HIV known as V3. This time, the animal produced plentiful neutralizing antibodies.

Researchers already known that V3 is vital for the virus to insert its genetic material into the host cell. If antibodies can be made to attach themselves to the loop, the virus could be disabled.

The second chimp received a different combination. First, it had a classical "vaccine" of inactivated virus, which produced only very few neutralizing antibodies. Second, the chimp received purified gp160, like the first animal. Again, it produced some antibodies, but few neutralizing ones. Finally, the second chimp also received the synthetic peptide from V3. This animal, too, produced plenty of neutralizing antibodies.

Both animals then received live HIV into their bloodstream. Six months later, the researchers have been unable to find any trace of the virus either in their lymph nodes or by amplifying the DNA from their cells using the polymerase chain reaction technique. This suggests that the inoculations have protected them - so far at least. Two control animals injected with HIV have developed symptoms.

However, Girard stresses the problems that remain. It is still possible that the animals are infected but that the team has failed to pick up signs of the virus. Secondly, and the biggest problem, the researchers do not know which inoculation or combination of inoculations has provided the apparent protection. Theoretically, each inoculation or combination of them should have been tested separately on separate animals but this is impractical, says Girard: the animals cost $40,000 each and are hard to come by.

The Pasteur Institute and its offshoot Pasteur Vaccines, which did much of the work, are

collaborating with the company Transgene in France and other centres in the US. Girard plans to try to repeat the tests and find out the role of V3. If this is successful, he will apply for permission to test the peptide in people. (Source: New Scientist, 5 May 1990)

Roche, Miles see AIDS advances on two fronts

Research scientists have reported two advances in therapies which may potentially be used to treat AIDS.

British scientists, including a team from Switzerland's Hoffmann-La Roche & Co., said they have synthesized more than a dozen new proteinase inhibitors. Proteinase is an enzyme by which the AIDS virus reproduces itself and numerous drug companies have focused their AIDS research on developing molecules to block the enzyme's action.

The British team claims their proteinase inhibitors are "of considerably enhanced potency", and potentially less toxic than other reported inhibitors.

The second report focuses on a calcium channel blocker, nimodipine, sold by Miles Inc. under the name "Nimotop". A team of neurological researchers, studying the effects of AIDS on calcium build-up in the brain have suggested this build-up is how the virus damages nerve cells and may underlie the dementia afflicting about two thirds of AIDS patients.

Though the researchers said their theory of the virus-induced calcium build-up and nerve damage is yet unproven, the experiments suggest further experiments should be done, aimed at eventually testing nimodipine on AIDS patients. (Source: Chemical Marketing Reporter, 23 April 1990)

Oral doses of alpha interferon absorbed through the mouth can alleviate symptoms of AIDS, according to D. K. Koech of the Kenyan Medical Research Institute (Nairobi). Interferon is normally not given orally because of the belief that it would be destroyed by the acids in the stomach. Koech claims, however, that wafers impregnated with alpha interferon can alleviate AIDS symptoms within weeks, restoring appetite, and reducing fever, fatigue, fungal infections, diarrhoea and swollen lymph nodes. Symptoms were alleviated within five weeks in all 99 patients tested. Other experts are skeptical that the treatment works. Injections of far higher doses of interferon have previously been ineffective. Further studies of the oral therapy will be conducted in the US and by WHO in several other nations. The longest any patient has been treated so far is six months. CD4 lymphocyte counts rose sharply.

J. M. Cummins of Amarillo Cell Culture says that animal studies on other diseases have offered some support for the effectiveness of oral interferon therapy. J. M. Hassett of Mount Sinai Hospital (New York, NY) says he doubts that the benefits of oral interferon will be as great as described by Dr. Koech, but even if the therapy only improves appetite or reduces fatigue, it will still be very useful. Trials at Mount Sinai on 35 patients could begin in May 1990. (Extracted from New York Times, 4 April 1990)

Trials continue on experimental AIDS drug

Project Inform, a San Francisco group, has been given permission by FDA to continue its clinical trials of compound Q, an experimental AIDS drug. In 1989 a doctor at San Francisco General Hospital announced that the drug, which is derived from cucumber root, kills AIDS-infected immune system scavenger cells but does not hurt uninfected cells. The report generated a great deal of excitement among people with AIDS and their advocates. Project Inform began importing compound Q from China for secret trials. Advocates for AIDS patients say the move was necessary in light of government regulations they regard as excessive. But when three people involved in the compound Q trials died, FDA took steps to block further testing. Some AIDS researchers are criticizing FDA's decision to allow the compound Q trials to resume. They accuse the agency of caving in to pressure from AIDS patient support groups and backing away from its responsibilities. FDA counters that it is simply trying to bring the compound Q trials into the formal clinical trials process. The new study will be done by the same private physicians who were involved in the secret trials. Genelabs, a subsidiary of Sandoz Pharmaceuticals, will supply a synthetic form of compound Q. (Extracted from New York Times, 9 March 1990)

Livestock applications

What to do with an animal map

Cattle will likely be the first farm animals for which researchers produce a genetic map. And although many of the participants at the recent Banbury Center meeting talk of the benefits of a cattle map in terms of dollars per steak, Alan Teale of the International Laboratory for Research on Animal Diseases in Nairobi has a different use in mind for the map, whenever it is done.

Teale does not want to produce a leaner steak. Rather, he wants to provide food for a protein-poor region of Africa. To do so, he wants to track down the genes that control resistance to trypanosomosis, better known as sleeping sickness.

Because of this tsetse-borne disease, some 10 million square kilometres in Africa are unsuitable for cattle. Eradicating the tsetse fly will be difficult if not impossible, and prospects for a vaccine are dim, says Teale. Short of those two options, resistant cattle would be a tremendous boon. Teale considers them "a backstop that might save you from catastrophe".

One breed of cattle, the N'Dama, are resistant to the parasite, but they are scrawny and are poor milk producers. Teale wants to use the genetic map now being developed by James Womak of Texas A&M University and others to find markers associated with the resistance so that he can introduce it into the popular Boran breed, though he admits at the outset that he has "absolutely no idea how many genes are involved".

Although the cow genetic map has been garnering the most attention so far, pig maps may well be next. At the University of Illinois, Larry Schook, Harris Lewin, David McLaren and Matthew Wheeler are just starting to look for markers with a grant from the National Pork Producers Council. They want to find the genes that control the huge litter size in the Chinese pig, a fat, homely animal reminiscent of

a Shar-Pei dog. The Chinese pig has 20 or so piglets per litter, as opposed to eight or ten in the litters of domestic animals, but the meat is too fatty. If the group can find markers close to the genes, then they can select for them to breed the large-litter trait into the domestic pig. (Source: Science, Vol. 248, p. 552, 4 May 1990)

Sericulture labour-saving technology using plant hormone

Daicel Chemical Industries Ltd., and the National Institute of Sericultural and Entomological Science have jointly developed a technology for stimulating silkworms to move up to their cocooning frames by using a plant hormone.

A cocooning frame is a corrugated board frame partitioned into square compartments the size of a photographic film box. Silkworms about to produce cocoons move up to this frame to do so. This is an important process in sericulture, but since it must be accomplished within one or two days, this has been a major problem in large-scale sericulture and for achieving labour-saving in the trade.

β-ecdysone was discovered in Japan in 1944, extracted in Germany in 1954, and its structure was elucidated in 1964. Feeding silkworms this hormone is known to stimulate them into moving up into the cocooning frame. This hormone, extracted from insects, is very costly, so it is not used in sericulture.

Daicel Chemical Industries and the University of California, Berkeley (USA) discovered that the roots of Vitex strickeri, an indigenous African shrub, contain a high concentration of β-ecdysone and established a technology for economically extracting the substance. At the same time, the company developed a technology jointly with the National Institute of Sericultural and Entomological Science for applying the substance to sericulture.

In experiments at the institute, it was confirmed that whereas only 80 per cent of the silkworms not doped with β-ecdysone climbed into the cocooning frame in 40 hours, those that were fed with leaves sprayed with 10 ppm of the substance all climbed into the cocooning frame in 20 hours. As a result, the work of moving the silkworms into the cocooning frame has been conspicuously alleviated, mass processing is now possible, cocoon quality deterioration due to long-unattended cocoons is prevented, and the yield has been substantially improved by increasing the turnover of moving the silkworms into their cocooning frames. Further details available from Daicel Chemical Industries Ltd., Research Centre, 1239, Shinzaike, Aboshi-ku, Himeji City, Hyogo Pref. Tel.: 0792-73-8524, Fax: 0792-74-4074. (Source: JETRO, May 1990)

DNA-probe tests used in turtle repopulation programme

DNA-probe tests have been used to distinguish the sex of newly hatched ridley sea turtles, in research by S. Demas at the University of Tennessee (Memphis). The tests are based on the DNA fingerprinting process. The probe in this test is a DNA fragment from a poisonous Asian snake which attaches to gender-specific DNA fragments. Small blood samples from the ridley turtles were used. The procedure correctly distinguished the sex of 29 out of 30 ridley turtles tested, and 9 out of 10 green sea turtles tested. Radioimmunoassay for testosterone is 90 per cent accurate for two-year-old turtles, but that would slow the ridley turtle repopulation programme of the National Marine Fisheries Service (Galveston, TX). The DNA test may also be used to understand the effects of incubation temperatures on the gender of turtle hatchlings. There are more female hatchlings when the incubation temperature is comparatively warm. Male hatchlings are more numerous when the incubation temperature is 5-8°C lower. (Extracted from Science News, 13 January 1990)

Transgenic carp: outdoor test

The first outdoor experiment with a genetically engineered, transgenic fish has received tentative approval from the US Department of Agriculture (USDA). Pending final public comments, the action clears the way for Auburn University researcher Rex Dunham to see whether carp containing a trout growth hormone gene can transfer the genetic trait from generation to generation and to monitor how the gene affects fish development.

At issue is whether the experiment would pose any environmental threat to Alabama streams. Dunham plans to stock 10 ponds with the fry from nine transgenic carp that will be spawned in a laboratory. The fish will be grown and studied for one year and then destroyed before they reach sexual maturity.

USDA's Office of Agricultural Biotechnology says the safety measures that Auburn has taken to make sure the fry do not escape into nearby streams are adequate. But this finding is being challenged by the National Wildlife Federation (NWF). NWF biologist Margaret Mellon, citing the presence of carp and other exotic fish in nearby waterways, questions Auburn's containment mechanism.

Moreover, she says the department's environmental assessment should be augmented with a more detailed environmental impact study (EIS) that includes the participation of other federal agencies such as the Department of Interior's fish and wildlife division. The organization may seek a court injunction to stop the experiment pending the outcome of an EIS. (Source: Science, Vol. 247, p. 1298, 16 March 1990)

The sweet way to build a bigger pig

Pig farmers could produce better, heavier animals that need fewer antibiotics with the help of a feed of combined sugars. The sugars work by nourishing beneficial bacteria that live in the pigs' intestines.

BioEurope, a French biotechnology firm, has developed the specialized food additive. It is a group of sugars which increase the pig's weight at market by up to 9 per cent. The company says the sugars could replace the much-criticised antibiotics that farmers now feed routinely to livestock to boost growth.

Farmed animals grow bigger and faster if they are fed antibiotics that work against a wide spectrum of bacteria, whether the animals are obviously sick or not. This practice, widespread among farmers, is thought to work by killing harmful gut bacteria, a source of much ill-health among crowded, stressed animals, though the drugs also kill beneficial bacteria. But the practice also breeds antibiotic-resistant bacteria, and leaves residues of the drugs in meat, much to the alarm of consumer groups.

BioEurope describes its sugars as "pre-probiotics". Probiotics are beneficial bacteria that normally inhabit the gut. François Paul, a researcher with BioEurope, says probiotics are beneficial to their host animals because they compete with coliforms and other bacteria such as Clostridium, which causes botulism and other infections. The probiotics reduce the numbers of such bacteria and help to digest some food, making more available for the animal. They can also stimulate macrophages, white blood cells in the gut which destroy harmful micro-organisms.

Some researchers have attempted to boost the numbers of probiotics in the gut by feeding them directly to animals - and humans - but their attempts have so far been disappointing, says Paul. He says, for example, that there is no evidence that the lactobacillus bacteria in live yoghurt join the colonies of similar bacteria already resident along the gut wall.

BioEurope's technique to boost probiotics involved designing a molecule that could survive its journey through the digestive tract to the colon, where the bacteria live. The molecule would then have to feed probiotics, but not harmful bacteria. The company's researchers used enzymes that link sugars together in ways that cannot be broken down by digestive enzymes in the saliva, stomach or small intestine, to create various chains of the simple sugars sucrose and glucose, known as oligosaccharides. Next, the team fed the oligosaccharide to cultures of gut bacteria, and selected those chains which could be digested only by probiotics.

The researchers then fed the oligosaccharides to pigs and compared the animals' growth with that of pigs in the same barn that were fed none of the sugars. After three months - the normal fattening period for farmed pigs - those on the pre-probiotic were on average 6 kilograms heavier than the control pigs, an increase of 9 per cent. (Source: New Scientist, 14 April 1990)

Agricultural applications

"Shaking up" genetic transformations in plants and insects

Using a dime's worth of silicon carbide whiskers and a commonplace laboratory mixer, an agricultural researcher has found a way to speed up genetic transformation of plants and insect eggs a thousandfold.

"The idea is simple. We put the insect eggs into a solution with the DNA that we want to introduce. Then we add something small, hard and sharp - the silicon carbide whiskers - and agitate it in a vortex mixer", says research geneticist Andrew Cockburn, at the US Department of Agriculture (USDA) Insects Affecting Man and Animals Research Laboratory here. The whiskers poke tiny holes in the eggs, allowing DNA to seep in.

Cockburn views this technique as an alternative to micro-injection, a painstaking method in which technicians take the embryos one by one and inject DNA into them.

The new technique, which Cockburn describes as "being in a jacuzzi with a porcupine", is also, he says, far less costly. "I have done hundreds of experiments with one free sample of silicon carbide whiskers, which would have cost about ten cents if I bought them. And a vortex mixer costs about $180," he estimates, adding that micro-injection equipment, which includes a good microscope, a micromanipulator and needle-maker, can cost about $15,000.

The USDA filed a patent for the technique, which has potential applications in genetically engineering crop plants as well as insect pests. (Source: McGraw-Hill's Biotechnology Newswatch, 19 March 1990)

Genetically uniform potato seed

TSP Partners has been formed by Escagenetics (San Carlos, CA) and Pioneer Hi-Bred International to produce genetically uniform potato seed. Because potato genes produce seed in a wide variety of combinations, farmers have had to sow dormant buds of full-grown potatoes, where the genetic combination is already fixed. Escagenetics' researchers have come up with parental strains of potato plants that produce genetically uniform seed. Escagenetics then formed a joint venture with Pioneer Hi-Bred, the large seed company. TSP Partners, the new formation, is now producing seed by the hundreds of kilograms. According to a company spokesperson, one kilogram can plant approximately 20 acres at a much cheaper cost than using cut potatoes. (Extracted from Wall Street Journal, 8 February 1990)

No quick fix for raising crop yields

Cereal crops which can "fix" their own nitrogen from the environment have become one of the holy grails of scientists working in agricultural biotechnology. If genetic engineers could alter the DNA of staple crops such as wheat and rice in this way, then farmers may no longer need to spread ton after ton of nitrogen fertilizer on their fields.

But by concentrating on nitrogen fixation the biotechnology industry is not promising to solve any food crises. Reducing the use of fertilizers would almost certainly cut costs for farmers, and may even help to protect the environment. But it is only in developing countries, where farmers cannot afford to use fertilizers today, that yields of crops could be expected to rise.

The biotechnology community is divided over whether crops which fix their own nitrogen would produce any more grain than those grown elsewhere in the developed world with an abundant supply of fertilizer. Many scientists believe that natural limitations on the photosynthesis which creates energy in plants will prevent the new technology from raising the yields of such crops.

Leguminous plants are well adapted to exploit nitrogen from the soil and air. They have a symbiotic relationship with bacteria which convert nitrogen into a form the plants can use. Barry Smith, from the AFRC's Nitrogen Fixation Laboratory at the University of Sussex, is trying to shift the 20 genes in these bacteria responsible for nitrogen fixation into the genetic blueprint of other crops.

This is very difficult. Biotechnologists can usually shift only one or two genes into new hosts. And, to date, the standard techniques of

genetic engineering have failed to incorporate any foreign genes at all into the cereal crops. Smith believes it will be at least 20 years before his work will produce a commercially useful crop.

Smith and his colleagues also acknowledge that these engineered crops will still "decide" for themselves the amount of solar energy they should convert into a useful form, by photosynthesis. This "photosynthetic rate" will limit the amount of energy available to the plant as a whole, because the crop will use some of the energy it converts to express the genes for nitrogen fixing. This energy will not therefore be available for swelling the seeds and increasing the yield of the crop.

Colin Law, head of the AFRC's Cambridge laboratory for plant breeding, believes that nitrogen fixing may only be one part of the puzzle of increasing yields. It may also be necessary, he says, to insert foreign genes to alter the photosynthetic rate of plants. "At the moment it is not clear what genes you need to put into plants to increase their yields. There are lots of unknowns."

Law is more optimistic about other ways in which biotechnology might help to increase yields. One example would be the use of molecular probes to select the best plants to breed from. This might help farmers to overcome "plateaux" in their yields - when no matter how much extra fertilizer they put on to their fields the yields of their crops stubbornly refuse to increase. Law also believes that biotechnology may help to increase the resistance of crops to their common pests and diseases.

John North, from the Department of Land Economy at Cambridge University and former head of the Government's agricultural advice service, is adamant that attempts to add genes conferring the ability to fix nitrogen will never increase yields from crops. North believes that in countries where there is no limit on the amount of nitrogen fertilizer available the crop will stubbornly photosynthesize only the energy it decides it needs. (Source: New Scientist, 31 March 1990)

New technology for panicum crop

A new cell culture technology using dedifferentiation/redifferentiation media for the autumn pasture crop panicum has been developed in research at the National Agricultural Experiment Station (Shikoku). The sterilized seed is first cultured in the dedifferentiation media for two months, followed by the redifferentiation media. The dedifferentiation ratio reached 38 per cent with 60 grammes per litre of glucose and 2 grammes per litre of hydrolised casein in the media. The addition of 2,4-dichlorophenoxyacetic acid as a growth regulator also improved production. Autumn panicum can grow in moist areas, making an excellent feed grain for rice-paddy use. (Extracted from Japan Chemistry, 8 March 1990)

One man's pesticide

Research in agricultural biotechnology aimed at developing crops with genetically-engineered resistance to chemical pesticides threatens to "entrench and extend the pesticide era", according to a scathing report* issued by a coalition of environmental, farm, church and consumer groups in the United States. Genetic engineering techniques should be used instead, the report argues, to reduce the dependence of modern agriculture on chemicals. But that, biotechnologists reply, is exactly what they are trying to do.

The report is especially critical of the use of more than $10 million of public funds over the past four years on research into herbicide tolerance.

The report identifies 58 research projects at 27 private companies and 21 public universities and research centres that, it claims, will increase agricultural dependence on chemicals which continue to poison farm workers and find their way into food and water supplies. But Alan Goldhammer of the Industrial Biotechnology Association, a lobbying and trade group of biotechnology companies, says that the critics play down the ability of research into herbicide tolerance to sustain environmentally sound agricultural practices. He argues that the Environmental Protection Agency would have regulatory control over any potential commercial products, and that no-one in industry wants to commercialize a herbicide-resistant product that will encounter regulatory controversy.

But Jane Rissler, a plant pathologist at the National Wildlife Federation, and one of the principal authors of the report, is not so sanguine about either the current state of regulations or the direction of industry. She believes the agri-chemical industry "needs to be taken to task on the betrayal of their promise" to use biotechnology to lessen dependence on chemical pesticides, and says the report is intended to draw the attention of the public to research which takes the industry in what she sees as "absolutely the wrong direction". The same argument was made last week by various members of the coalition of public interest groups at three separate press conferences. (Source: Nature, Vol. 344, 29 March 1990)

Food production and processing

Pfizer given go-ahead to market first recombinant food ingredient

The US Food and Drug Administration (FDA) has given Pfizer Inc. the go-ahead to sell the first genetically-engineered food ingredient, a bioengineered copy of rennin. Analysts see the decision giving Pfizer a head start over competitors, such as Gist-Brocades, which aim to enter the market for recombinant milk coagulants. The new product promises better quality control in cheese production.

Pfizer uses Escherichia coli K-12 to produce the genetically-engineered rennin, which is identical to the natural enzyme. The bacteria are fermented at a new plant near Terre Haute, Indiana. It took the FDA 29 months to clear the product. (Source: Biotechnology Bulletin, Vol. 9, No. 3, April 1990)

Chemical industry applications

Extraction and purification of catechin from tea

The National Research Institute of Vegetables, Ornamental Plants and Tea has developed an efficient method for extracting and purifying catechin from tea leaves or processed tea for use as a food additive.

Catechin, a kind of organic substance known as a polyphenol compound, belongs to the same family as

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* Biotechnology's Bitter Harvest: Herbicide-Tolerant Crops and the Threat to Sustainable Agriculture, Biotechnology Working Group, March 1990.

the tannin in tea leaves. It is also found in grape seed. Featuring antibacterial, oxidation prevention, deodorizing, and other properties, it seems promising for use as a natural additive in food.

The volume of tea disposed of due to its lack of commercial value amounts to roughly 10,000 tons/year, so research is in progress to extract catechin for its commercialization; but since the separation of caffeine in tea leaves is quite difficult, there was a need to develop a selective catechin extraction and purification technology.

By using 80 per cent ethanol as an extraction solvent, the institute succeeded in extracting catechin from tea.

Tea extracts with ethanol, which were adsorbed on to a polymeric adsorbent resin or lipophilic gel filtration material, were washed with water, then extracted with an organic solvent containing water. Specific adsorption of catechin onto the gel filtration material occurred.

Leaching of caffeine and other impurities, except catechin, was performed by washing the gel onto which the tea extracts were adsorbed with a 15 per cent ethanol-water solution. The residue was extracted with an 80 per cent ethanol-water solution, and a catechin mixture containing few impurities was obtained.

The impurity content in the catechin mixture was less than 3 per cent, and the caffeine content was 0.1 per cent. The recovery of catechin was 57-73 per cent of the content of catechin in tea, depending on the amount of loading onto gel. Further details available from the National Research Institute of Vegetables, Ornamental Plants and Tea, the Ministry of Agriculture, Forestry and Fisheries, 2769, Kanaya, Kanaya-machi, Haibaragun, Shizuoka Pref. Tel.: 0547-45-4101. Fax: 0547-46-2169. (Source: JETRO, May 1990)

Energy and environmental applications

Synthesis of biodegradable plastic raw materials

Headed by Professor T. Nakahara, a research team of the University of Tsukuba has succeeded in using microbes to synthesize 4-hydroxybutyric acid, the raw material for producing a biodegradable plastic. The starting material is 1,4-butandiol mass produced as a raw material for manufacturing polyethylene. Adding the microbe into a solution containing this material converts nearly 90 per cent of the butandiol into hydroxybutyric acid in 24 hours. Since virtually no energy is required, the material can be produced at a much lower cost than by existing chemical synthesis processes.

Most microbes have the property of storing granular polyesters inside their bodies, and the biodegradable plastic is produced by using this polyester that is later dissoved with ease by microbes existing in the ground. The 4-hydroxybutyric acid, produced by a microbe, Candida rugosa, is used as the raw material for the biodegradable plastic.

The microbe is first cultured in a liquid containing nutrients, then transferred to a separate container containing 1,4-butandiol. In the experiments, a solution containing 50 g/l of 1,4-butandiol provided 50 g of 4-hydroxybutyric acid, a molar yield of roughly 90 per cent.

The raw material 1,4-butandiol is a common chemical in which hydroxy groups are bonded to both terminals of butane and is available at a low cost. The microbe does not multiply in the process of hydroxybutyric acid production, but can be multiplied with ease in the preceding culture process. Further details available from University of Tsukuba, Institute of Applied Biochemistry, 1-1-1, Tennoudai, Sakura-mura, Niiharigun, Ibaraki Pref. (Source: JETRO, May 1990)

Biodegradable plastic hits the production line

The United Kingdom's largest chemicals company, ICI, has announced the launch of the first practical plastic that is totally biodegradable. The material, called Biopol, took 15 years to develop and Wella, an international hair-care company, plans to begin packaging shampoo in bottles made from it this month. The bottles will be available only in Germany.

Warner Lambert, a chemicals company in the US, announced in February that it has developed a biodegradable plastic made from starch, although it is not yet in a usable form. Many companies claim to make plastics that are biodegradable, but the parts that degrade in these products are mounted on lattices of non-biodegradable plastic.

ICI has used a natural polymer called polyhydroxybutyrate (PHB) which degrades to form carbon dioxide and water. The rate at which fungi and bacteria break down the material varies, but it can disappear totally within weeks. The company obtains the material from a common bacterium, Alcaligenes eutrophus, which stores PHB in the same way that humans store fatty tissue. The company puts the bacteria into vats containing a broth of glucose and essential nutrients, where they make the PHB.

The amount of carbon dioxide that is released as the material degrades matches the amount extracted from the air by the plants providing the glucose, says ICI. This means that there is no overall increase in carbon dioxide, a major contributor to global warming.

Scientists can control the properties of Biopol by adding fixed amounts of a simple organic acid to the glucose. This generates a series of so-called copolymers which are mixtures of PHB and another compound, hydroxyvalerate. David Barstow, the Biological Products Business Manager at ICI, expects that the company will be able to supply a family of biodegradable plastics suited to particular applications.

The company, which manufactures Biopol at Billingham in north east England, plans to increase production to between 5,000 and 10,000 tons per year by the mid-1990s. (Source: New Scientist, 5 May 1990)

Bacterium that secretes excellent water absorbent substance

Dr. R. Kurane, Head of the Bioconversion Laboratory of the Fermentation Research Institute, has discovered a soil bacterium, Alkaligenus latus, that secretes a substance that can absorb a large volume of water, like synthetic polymers currently used for sanitary products and paper diapers. The newly discovered substance absorbs up to 2-5 times more water than synthetic polymers and it is biodegradable, so it does not cause environmental pollution.

When cultured in a medium containing minerals and various organic substances, the bacterium secretes a water absorbent substance that makes the water look as if it contains dissolved starch. Purifying, drying, powdering, and analysing the substance showed that it consists of a type of polysaccharide; and a survey of its water absorbency proved that it absorbs about 1,000 times more water than its own weight, which is about five times greater than that of commercially available water absorbent acryllic polymers which absorb 200-400 times more water than their own weight at most. In salt water, the substance's water absorbency is 400-500 times its own weight, or 5-20 times greater than that of synthetic materials. Moreover, the substance is also superior to the current synthetic polymers in absorption speed and water retentivity in dry environments.

The institute is presently conducting experiments on the bacterium's culturing conditions to enable it to be cultured in tanks, and studying other uses for the secreted substance.

The Ministry of International Trade and Industry is conducting an experiment in Egypt to prevent soil aridity by using water absorbent polymers with the objective of greening deserts. The synthetic materials used in the experiment resist decomposition and flow out from the soil into rivers to cause environmental pollution, however. The substance secreted by the newly discovered bacterium is readily biodegradable and is therefore much safer. Further details available from Fermentation Research Institute, the Agency of Industrial Science and Technology, 1-1-3, Higashi, Tsukuba City, Ibaraki Pref. Tel.: 0298-54-6024. Fax: 0298-54-6009. (Source: JETRO, May 1990)

Biotreatment to clean up creosote

UK company Biotreatment is attempting the in situ clean-up of creosote-contaminated soil using Pseudomonas bacteria. It is the first time the technique has been tried on creosote, and the first time with the soil still on site.

The clean-up will cost some $1.6 million and is taking place in Stockholm, on land that once housed Sweden's first gasworks. It is now scheduled for apartment blocks, but plans were postponed after the creosote was discovered. The Swedish National Environment Protection Board is contributing $320,000 to the project and will be assessing the results closely.

The area to be cleaned covers 5,000 m^2 and is full of creosote to a depth of 2-5 m. The polluted area has been boxed in with walls of sheet steel piles encapsulated in concrete. A layer of clay underneath the soil prevents the creosote from escaping, and a "lid" for the area has been made from aluminium foil and stainless steel covered with concrete. The Pseudomonas were injected by pipe in April.

The soil will be tested in two years' time, by which time the ground should be fit to be built on. The bacteria should reduce the creosote to carbon dioxide and water, which will be evacuated through a pipeline system. (Source: European Chemical News, 7 May 1990)

BioEnergy plans scale-up of biomass ethanol process

BioEnergy International LC has licensed a genetically-engineered bacterium which it says can produce ethanol from biomass such as agricultural wastes, wood and garbage at half the cost of traditional corn-based processes.

The exclusive world-wide licensee of the University of Florida Research Foundation Inc., BioEnergy plans to commercialize a recombinant version of E. coli, which can efficiently produce ethanol from five-carbon sugars.

Development of the organism was announced two years ago by Lonnie O. Ingram, a microbiologist at the Institute of Food and Agricultural Science at the University of Florida, but it was not until recently that increased efficiency and tolerance to alcohol has made it competitive.

BioEnergy says it will embark immediately with a pilot programme on selected feedstocks, using strategic partnerships - one of which should be announced shortly - to transfer the technology on an international scale.

Traditional ethanol production employs yeast to consume the six-carbon sugars and starch present in the edible portion of the corn plant. However, according to BioEnergy president, Thomas Hayes-Morrison, until Dr. Ingram's breakthrough, there was no way to utilize the inedible portion of corn and other feed crops.

This fibrous fraction, known as roughage, is composed about half of cellulose - a polymer of six-carbon sugars - and half of hemi-cellulose - a polymer of five-carbon sugars. Because of the presence of the five-carbon sugar, traditional yeast fermentation is ineffective on roughage.

Five-carbon sugars are, however, the basis of other chemistries and are used in the production of furfural and xylitol.

BioEnergy's five- and six-carbon production process is about as efficient as the traditional six-carbon yeast-based route, with the advantage that feedstocks are low cost, free or even negatively priced, since otherwise they are often disposed of as waste.

A huge potential feedstock pool for the process exists in agricultural waste - for example corn stalks, leaves and cobs; rice straw and hull; and sugar cane bagasse - that is currently burned, buried or mixed into cattle feed.

A feedstock source with more immediate commercial potential is waste streams from food processing facilities. Mr. Hayes-Morrison believes that within two years his firm could have a commercial operation at a food plant producing anywhere from 1 million to 10 million gallons of ethanol annually, depending on the type of waste stream.

The plant would be, in effect, bioremediating the customers' waste at the same time it produces ethanol, either for sale into the merchant market or for internal consumption as a fuel source.

This approach would also be effective, Mr. Hayes-Morrison says, at replacing existing waste treatment processes in pulp and paper plants and sugar mills. (Source: Chemical Marketing Reporter, 30 April 1990)

Underwater coal mine canaries

Biological monitoring is by no means new. For example, canaries have been deployed for centuries to sense toxic gases in coal mines. In general, any organism of appropriate sensitivity can function as an effective threshold probe. And although biological sensors lack certain specificity offered by physicochemical analysis, they have the advantage of immediate response, a first clue to the biological effects of unwanted chemicals. Can a small, invertebrate sea organism monitor water quality as efficiently and reliably as conventional chemical techniques? By way of a watchdog system, it can, says the Dutch Organization for Applied Scientific Research, TNO. Since last year, TNO, together with two research institutions and the company Delta Consult, have been experimenting with an "early warning system" based on living mussels, one that seems to tell instantly when water quality deteriorates.

The success of the "Mussel Monitor", as the system is called, rests on a familiar behaviour pattern of the Bivalva or two-valve molluscs: they shut their shells (valves) when the ambient oxygen level drops or when the concentration of toxic solutes in the water reaches a critical level. Under normal conditions, the mussels pump water through their gills to breathe oxygen and clam up, so to speak, only occasionally. The trick of the monitoring system consists in faithfully recording the valve movements of a set of animals submerged at a particular probing spot.

Each tell-tale kit consists of a 30-kilogram, cylindrical, watertight stainless steel case crammed with electronics - attached to a cage containing eight mussels. These - either the common marine species Mytilus edulis or the freshwater-dwelling Dreissena polymorpha - each have high-frequency electromagnetic induction sensors attached to their valves. The submerged mussels in the "Monitor" look like isolated ears, pricked to capture music supplied by headsets. Of course, the signals are going the opposite direction from a Walkman: the sensors connect to a small computer that tracks the closing times of the valves. If most of the mussels (e.g., six out of eight) keep their valves shut over a prolonged period (say five minutes or more), an alarm is activated.

Laboratory experiments have conclusively shown that a set of eight mussels can function effectively as such an early warning system, with response time as low as a few minutes. Performance tests indicate a high sensitivity for several important trace pollutants. Detection limits for zinc and lead are below 500 micrograms per litre; cadmium and selenium are found at concentrations below 100; copper and chlorine below even 10 micrograms per litre. The system is now operating in coolwater systems, both at the Maasvlakte near Rotterdam and at Dow Benelux. Delta Consult expects to market the device in spring 1990: "We believe there is a market for several hundred world-wide," says manager F. P. Montauban. At a price of 25,000-28,000 Dutch guilders (ECU 10,870-12,000), "If we sell about a hundred units, we will have recouped our costs." Apart from the "Mussel Monitor", TNO is also using a long-term, mussel-based system in which molluscs act as a living filter. Buckets containing a hundred healthy mussels are suspended for six weeks at sea; the animals pass 200,000 litres of water. In the process, toxic compounds accumulate in their tissues and post-mortem analysis reports on pollutive trends. This system operates along the Dutch coast, especially near the estuaries of the Rhine and other rivers. Further information: Dr. Kess J. M. Kramer, Laboratory for Marine Research, MT/TNO, PO Box 57, NL-1780 AB Den Helder. Tel.: (2230) 32924, Fax: (2230) 30687. (Source: Scientific European, April 1990)

Industrial microbiology

Biotech cellulose scales up

US-based Weyerhaeuser, in conjunction with biotechnology company Cetus, has developed a bacteria to produce a commercially useful form of cellulose fibre. The material, called Cellulon, is available in pre-production quantities, with commercial prices anticipated in the $6-10/lb range.

The cellulose is produced as an intricately crosslinked, fine fibre network, rather than the individual fibres of distinct length common to natural forms. This, plus the fibres' thinness (typically 0.1 μm width) and hydrogen bonding capability, make it suitable for thickening liquids at low concentrations and for coating paper. Weyerhaeuser is looking for uses in the oil, food, cosmetics, paint and ink markets. US FDA food-use approval will be sought once data are fully developed.

Cetus has developed a strain of Acetobacter bacteria for Weyerhaeuser that can produce the cellulose in large-scale commercial production. The companies had to overcome the problem of the bacteria being adversely affected by the shear and agitation required to get oxygen to them in large tanks. Another problem, the tendency of the species to produce significant amounts of byproduct acids, was overcome by mutagenesis.

Production is currently proven in fermenters up to 50,000 gal. capacity. Weyerhaeuser points out that to date most development work has been on small-scale static bacterial production, resulting in high-cost cellulose material. Other companies in the field include Ajinomoto, ICI and Bio Fill Productos Biotecnologicos of Brazil. (Source: European Chemical News, 30 April 1990)

E. PATENTS AND INTELLECTUAL PROPERTY RIGHTS

Ecogen files patent for corn rootworm biopesticide

Ecogen Inc. has filed a US patent application for a purified, crystalline protein that has insecticidal activity against the corn rootworm. The insecticidal crystal protein (ICP) was isolated from a novel strain of the bacterium Bacillus thuringiensis (Bt). The patent application covers the ICP, the gene which governs the production of the ICP and Bt strains which contain the gene. Ecogen researchers discovered the corn rootworm activity of the Bt strain as part of a research collaboration with the Monsanto Company and subsequently cloned and sequenced the ICP encoding gene. Ecogen has granted Monsanto an exclusive licence to use this gene in corn plants and certain bacteria. According to US Department of Agriculture estimates, pesticides used to control corn rootworm comprise the single largest insecticide market in the USA. The insecticides are applied to the soil at planting time to prevent the corn rootworm from feeding on the underground stem and root system of the corn plant. Details from: Ecogen Inc., 2005 Cabot Boulevard West, Langhorne, PA 19047-1810, USA. (Source: Biotechnology Bulletin, Vol. 9, No. 3, April 1990)

British Library's patent express offers Currentscan

Currentscan keeps a watch on patents published by any of the major countries of the world. Copies of patents fitting a pre-set profile can be despatched to subscribers by fax, courier or first class post. Details from Currentscan, Patent Express, 25 Southampton Buildings, London WC2A 1AW, UK.

Patent information

It is estimated that approximately 85 per cent of technical information found in patent specifications is not published in any other form. Furthermore, even where there is another publication, it frequently occurs after publication of the patent specification. Therefore patent specifications represent an enormous technical resource, which is very poorly used by research workers other than those in commercial laboratories. Although this large body of information is available, and can readily be accessed by computer data bases such as Derwent's World Patent Index and World Patent Index Latest, it can be difficult to obtain full value from searchers unless the searcher is familiar with the international classification system, and the way in which information such as date of publication, etc., is indexed. The British Library has now published a guide to people who need to search for information in the patent literature, entitled "Introduction to Patents Information" by Stephen van Dulken. The book explains the international classification system, the codes used by patent offices, different methods available for searching by name or subject matter, and provides a bibliography on all aspects of industrial property protection. Details of the publication, priced at £20, from: Publications Sales Unit, Document Supply Centre, Boston Spa, Wetherby, West Yorkshire, LS23 7BQ, UK. (Source: ABA Bulletin, Vol. 5, No. 2, April 1990)

Plant variety rights

Following accession by Poland, there are now 19 member States of the International Union for the Protection of New Varieties of Plants. The member States are as follows: Australia, Belgium, Denmark, France, Federal Republic of Germany, Hungary, Ireland, Israel, Italy, Japan, Netherlands, New Zealand, Poland, South Africa, Spain, Sweden, Switzerland, United Kingdom and USA.

Several organizations have advised the Plant Variety Rights Office of their interest in carrying out plant variety rights growing trials on behalf of applicants, and a list may be obtained from the Plant Variety Rights Office. These organizations include seven Australian and one overseas bodies, and include State Departments of Agriculture, CSIRO, university departments and commercial bodies. (Source: ABA Bulletin, Vol. 5, No. 2, April 1990)

Patentability and convention priority in the United States

The US Patent and Trademark Office Board of Patent Appeals and Interferences has upheld rejection of claims directed to "human NGF comprising the amino acid sequence ... and which is free of other proteins of human origin". The inventors had synthensized NGF using recombinant DNA technology, and therefore it was free of human proteins which would otherwise be expected to contaminate the preparation. Prior publications disclosed what appeared to be purified B-human NGF. The Examiner stated that the claimed invention was prima facie obvious, and that the applicant therefore had to demonstrate that at the priority date there was no known or obvious method of making the claimed composition, or that the claimed composition possesses unexpected characteristics. It was held that neither of these facts was established by the evidence.

The fact that the inventors were the first to clone the gene for NGF and to determine the amino acid sequence of NGF was not sufficient to demonstrate patentability of the claims.

This case represents the US Patent and Trademark Office's current position concerning allowance of such claims. Therefore, where a previously known protein has been cloned for the first time, it will not be possible to obtain a patent for the protein itself; however, it may be possible to obtain claims to the method for production of the protein, and to the protein as produced by that method. (Source: ABA Bulletin, Vol. 5, No. 2, April 1990)

"Use" claims and "second medical use" claims to be allowable in Canada

Following two recent court decisions, in which it was held that a new use of a known compound or composition is patentable, and that a specific use of a novel compound or composition is also patentable, the Canadian Patent Office will now accept claims of the following types:

(a) When the invention is a novel compound X or a novel composition Y:

1. Compound X (composition Y) for the use of ...

2. The use of compound X (composition Y) for ...

3. The method of using compound X (composition Y) comprising ... (set forth various steps of the method).

(b) When the invention is a known compound A (composition B):

1. Compound A (composition B) for the (new) use of ...

2. The use of compound A (composition B) for (the new use).

Where the use of the compound or composition is a medical one, claims of types 1 and 2 above will be allowable, but not claims of type 3, because the latter would define non-allowable methods of medical treatment.

Because of the prohibition of claims for methods of medical treatment, the Canadian Patent Office has previously only permitted claims for second medical use in the highly restricted European form, and there has been some doubt as to whether the courts would uphold even these. Thus Canada now becomes much more attractive as a country in which to file patent applications involving second medical use inventions. (Source: ABA Bulletin, Vol. 5, No. 2, April 1990)

Common pool of patent information to be established

The US Patent and Trademark Office, the Japanese Patent Office and the European Patent Office have signed a memorandum of understanding

which commits them to develop a common standard for the electronic coding of patent documents, and will create a common pool of patent documents which will be instantly available from any of these three offices. This will enable searchers in one country to access patents from another, via computer and modem. The eventual aim is that all countries will conform to the common coding standard, and specifications will be available on the same day in all countries. Abstracts of European and Japanese specifications will be available in English, and will enable quick access by key word searching. The European and US Patent Offices will issue their data base on CD ROM discs, and these will be made available to the public on a trial basis. (Source: ABA Bulletin, Vol. 5, No. 2, April 1990)

New requirements for biotechnology patent applications

Because of the complexity of patent applications dealing with nucleic acid or amino acid sequences, Patent Offices have had great difficulty in carrying out searches in order to determine whether a given sequence is novel. Although they have made use of the major existing data bases, it is still difficult to compare whether two sequences are related. Hitherto there have been no standards for the representation of sequence data in patent applications, and applicants have used a variety of sequence descriptions and representations. Following detailed discussions between the US Patent and Trademark Office, the European Patent Office, and the Japanese Patent Office, these organizations have decided to adopt a standard format for presentation of sequence data, which will be compatible with the six major non-patent data bases, and which will be machine readable.

Effective 1 January 1990, the US and European Patent Offices are adopting new rules, the latter being on a trial basis. From 1 April 1990 applicants to the European Patent Office are strongly recommended to use the new format, which will eventually become mandatory.

The new rules state that patent applications which disclose a sequence of four or more amino acids, or a sequence of 10 or more nucleotides, must contain the sequences in computer readable form, using machine-readable OCR characters. Standard terminology must be used. For US applications, applicants will be required to submit a computer-readable diskette or tape containing the relevant sequences, which will also help in accurate printing of the patent specifications as issued. The US office will accept the following computer/operating system configurations:

1. Computer: IBM PC/XT/AT, IBM PC/2 or compatibles
   Operating system: PC-DOS or MS-DOS (versions 2.1 or above).

2. Computer: IBM PC/XT/AT, IBM PC/2 or compatibles
   Operating system: UNIX or XENIX System V.

3. Computer: Apple MacIntosh
   Operating system: MacIntosh.

The European Patent Office has stated that if there is sufficient demand, it will make available a diskette with an input programme (entitled "AUTHORIN") which elicits all the information required in a prepared, standardized form, which follows as closely as possible the submission form used by the six major non-patent data base producers. This diskette will also be distributed by the US Patent and Trademark Office.

A copy of the notification appeared in the Official Journal of the European Patent Office on 18 December 1989, as a supplement to Official Journal 12/1989. (Source: ABA Bulletin, Vol. 5, No. 2, April 1990)

New US Patent Office rules on deposit of biological materials

New rules regarding deposit of biological materials came into effect in the United States on 1 January 1990. Most of these rules clarify previously existing procedures. Their key points are as follows:

(a) A biological material is defined as "any material that is capable of self-replication either directly or indirectly".

(b) A biological material need not be deposited if it is known and readily available to the public, or can be made or isolated without undue experimentation.

(c) Deposits may be made in any International Depositary Authority established under the Budapest Treaty, or any other depositary recognized by the US Patent and Trademark Office.

(d) A deposit may be made before the filing of a patent application, or while it is pending. Deposit before filing the patent application is recommended. (NB: for the purposes of other countries, it is necessary to make the deposit before the earliest priority data of the application.)

(e) If a deposit becomes contaminated or has lost viability or function, it may be replaced either while the application is pending, or after the patent has been issued. It may be necessary to show diligence in making the replacement deposit.

(f) The deposit is made for at least 30 years, and at least five years after the most recent request for the furnishing of a sample by the depositary.

(g) A viability test must merely demonstrate that the deposited material is capable of reproduction; it is necessary to demonstrate that it can perform any of the functions described in the application.

(h) The conditions of deposit must assure that the Patent and Trademark Office will have access to the deposit while the application is pending, and must assure that all restrictions imposed by the depositor on the availability to the public of deposited material will be irrevocably removed on grant of the patent. (NB: for the purposes of other countries, samples of the deposit must be available following publication of the application).

(i) If during examination of the application the Examiner determines that a deposit is needed, and has not been made, or that the deposit does not comply with the regulations, the Examiner should reject the affected claims, with explanation of why a deposit is needed and/or why a deposit already made cannot be accepted. The applicant can then

respond to such a rejection by making an acceptable deposit, or by assuring the office that an acceptable deposit will be made on or before payment of the issue fee; alternatively, the applicant may argue why a deposit is not necessary under the circumstances. If an application is otherwise in condition for allowance, and the office has received a written assurance that acceptable deposit will be made on or before payment of the issue fee, it will issue a Notice of Allowance and issue fee due, together with a requirement that the necessary deposit be made within three months.

For each deposit made in accordance with the new guidelines, the specification must contain the following information:

1. The accession number of the deposit.

2. The date of the deposit.

3. The name and address of the depositary institution.

4. A description of the deposited material sufficient to identify specifically, and to permit examination. (Source: ABA Bulletin, Vol. 5, No. 2, April 1990)

Stanford patents pay

Licensing genetic engineering technology is becoming more lucrative by the day for Stanford University. The institution estimates that since 1981 it has earned US$17 million alone on a single three-part patent covering gene cloning techniques.

Developed back in 1973 by Stanford geneticist Stanley N. Cohen and Herbert W. Boyer, a chemist at the University of California at San Francisco, their patented discoveries accounted for 42 per cent of Stanford's technology licensing income in 1989. The institution has just issued its 100th licence covering the rights to the gene cloning methods to Henkel Research Corporation.

Stanford currently is charging companies with 75 or more employees a US$50,000 sign-up fee, requires a US$50,000 annual payment, and imposes a 2 per cent royalty on end products that rely on patents held by the institution. (Source: Science, Vol. 247, p. 1,298, 16 March 1990)

F. BIO-INFORMATICS

Biotechnology marketing sourcebook

This is a new publication from the Biotechnology Information Service at the British Library. It lists over 250 English language periodicals, newsletters and abstracts of interest to those working in the life sciences, biotechnology, health care, biochemistry and related fields. Price: £30.00. Details from: Paul Wilson, Marketing and Public Relations, British Library, 25 Southampton Buildings, London WC2A 1AW, UK.

Chinese biotechnology market

A report compiled by the Division of Biological Sciences of the Chinese Academy of Sciences, Biotechnology in the People's Republic of China, is now available. It covers the agencies, institutions and universities involved in biotechnology. And it predicts that the biotechnology industry market in China will be worth over $14 billion by the year 2000. Details of the report, priced at $80.00, from: Han Yingshan, WIBIO, Chinese Academy of Sciences, P.O. Box 74006, Moshan, Wuhan 430074, People's Republic of China.

Biotechnology directory - new edition

The ABA recently completed all material for the second edition of the Australian and New Zealand Biotechnology Directory and it is now in the hands of the printers. Australian Industrial Publishers are once again publishing this Directory in conjunction with the ABA and will handle all marketing as in 1989. Australian Industrial Publishers expects to be releasing the Directory in May. Prices have been reduced for the second edition to $55.00 for ABA members and $65.00 for non-members (plus $5 postage and packaging). For a Directory of this quality, it is certainly good value at this price.

For those of you who did not see the first edition, first of all you missed out on a really good working tool which should be on everyone's desk; and secondly, you will have known nothing of the contents. The Directory contained 150 pages packed with information on companies working in biotechnology-related areas, and on organizations in biotechnology, not only in Australia and New Zealand, but also in Asia. There were also a number of specialist sections on matters such as Government contacts, training courses and microbial culture collections.

The new second edition contains all this and more. The classified section on types of companies and areas of business they are in has been completely revamped and put into a much more usable format. The information on R&D in Asia has been extensively revised, enlarged and improved. The section on Government contacts and contacts for registration of products has also been enlarged and improved. The section on training courses in biotechnology has expanded dramatically in this second edition. (Source: ABA Bulletin, Vol. 5, No. 2, April 1990)

Feeding tomorrow's world

Will the benefits of the "green revolution" of the 1960s and progress in food production meet the needs of the world population at the end of this century? Will the "biotechnological revolution" offer a solution to world food problems? How can aid to agricultural development and self-sufficiency be attained, particularly in developing countries?

In Feeding Tomorrow's World, Albert Sasson of UNESCO tries to answer these and many other pressing questions through a pluridisciplinary approach to human nutrition and food production. The book, addressed to a wide readership, provides a remarkable synthesis of the scientific, economic, socio-economic and environmental aspects of nutrition throughout the world.

Subjects treated:

Human nutrition

Nutritional needs
Protein deficiency and malnutrition
Nutrition and infection
Children's diet
Changes in diet and attitudes towards food
Diet and health

Production and trade in agricultural foodstuffs

Problems of evaluation and diagnosis
Changes in food production
Factors affecting food-production patterns
Effects of climatic variation on production
Regional and national situations
Modalities of production, acquisition and use of agricultural commodities in the developing countries
International trade in agricultural commodities

Achievements and potential; international co-operation and prospects

Hunger and poverty
Requirements for rural development
The "green revolution"
Agriculture, agro-forestry and livestock husbandry: international agricultural research centres, regional and international co-operation, transfer of results
Conservation and utilization of plant genetic resources
International assistance and co-operation
Prospects

Feeding Tomorrow's World (Sextant, 3), 1990, 805 pp. ISBN: 92-3-102083-8. FF 225.00; $US 53.00; $Can 56.50; £34.00. Co-published with the Technical Centre for Agricultural and Rural Co-operation (CTA), "De Rietkampen", Galvanistraat 9, 6716 AE Ede, Netherlands. Worldwide sales rights: UNESCO, 7 place de Fontenoy, 75700 Paris, France.

Biotechnology of vitamins, pigments and growth factors
Elsevier Applied Biotechnology Series
edited by E. J. Vandamme, Laboratory of General and Industrial Microbiology, State University of Ghent, Copure Links 653, B-9000 Ghent, Belgium.

Comprehensive coverage of the microbial synthesis and production of all economically important vitamins and several key pigments and growth factors is presented for the first time in this authoritative handbook.

An international group of contributors from Japan, Europe, Australia and Israel stress how deeply biotechnology - based on the action of bacteria, yeast, fungi and microalgae - is involved in the production of vitamins and related compounds.

The introductory chapter sets out basic information on vitamins and their economic production. Then follows a complete and systematic overview of the biosynthesis of all water-soluble and fat-soluble vitamins, pigments and special growth factors, including ATP, polyunsaturated fatty acids, gibberellins and co-enzymes.

Each chapter has data on discovery, chemical and physical properties, production by microbial or algal strains, screening, biosynthesis and regulation, strain improvement and genetics, fermentation or bioconversion processes, recovery and purification, (bio)assay methods, biological properties, chemical synthesis, formulation, applications and economics.

Given this wide scope, the book will benefit biotechnologists, biochemists, biologists, microbiologists, nutritionists, organic and analytical chemists, physiologists, pharmacologists, fermentation specialists, etc., both in research and academic institutions and throughout the process industries.

Copious illustrations, formulae, tables and flowsheets feature in each chapter, as does an up-to-date bibliography. Price £68.00/$US 122.50. Available from Elsevier Applied Science, Crown House, Linton Road, Barking, Essex IG11 8JU, UK. In USA and Canada: Elsevier Science Publishing Co. Inc., P.O. Box 882, Madison Square Station, New York, NY 10159.

Monoclonal antibodies - an international market analysis

This new report analyses the evolving monoclonal antibody markets. The MAb markets may well be the most dynamic of the healthcare industry; the technology and product applications seem unending.

The study is designed to provide marketing and business executives with market data and corporate profiles (not technical information). The study covers market issues with a primary focus on new developments and trends. This includes the markets for monoclonals used in immuno-conjugate, in-vitro diagnostic and therapeutic applications.

An example of the dynamics of the MAb markets is their use in therapeutics. Using monoclonals as drug carriers will enable drugs to be targeted to very specific locations. This method will be useful in treating cancers, cardiovascular diseases, anti-inflammatory and anti-infective conditions. While the current market for monoclonals in therapeutic applications is nil, by 1994 worldwide sales will be over US$550 million. And this market is expected to grow strongly into the early 2000s.

In total, market data are provided for 16 different monoclonal antibody applications. These applications include in-vivo diagnostics such as malignant melanoma, colorectal cancer, ovarian cancer, lung cancer, breast cancer, prostate cancer, stomach cancer, pancreatic cancer, heart attacks, deep-vein thrombosis, strokes, pulmonary embolism and atherosclerosis; in-vitro diagnostics; research and therapeutics. Each of these 16 applications are evaluated for future potential separately for the United States, Western Europe and Japan.

These data are summarized in 57 tables. Each table provides the projected market to the year 1994 as well as current sales.

A separate section of the report provides corporate profiles of 92 companies involved in the development of monoclonals. This includes companies in the US, Canada, Western Europe and Japan. Each corporate profile includes MAbs currently being developed and joint ventures for development. Report No. 981, December 1989. 111 pages, 57 tables, 92 corporate profiles, US$795.

Diagnostic imaging equipment

Theta has published a report which analyses the growing US market for diagnostic imaging equipment - magnetic resonance imaging, computerized tomography, positron emission tomography, mammography, digital substraction angiography, digital radiography, radiographic/fluorographic, radiographic X-ray, mobile image intensifiers, mobile X-ray and portable X-ray.

The world-wide market for diagnostic imaging equipment reached US$6 billion in 1988. Much of that growth can be attributed to the success of MRI. Sales of MRI units in 1988 reached 385 for a dollar volume of US$665 million.

The market for PET scanners will also grow strongly. Although the PET market was stagnant until 1988 with unit sales of only nine that year, Theta predicts, based on PET's effectiveness in cardiology, that during the next five years the market will grow at an average rate of 54 per cent reaching a volume of 50 units per year by 1993.

The report includes 53 tables and 22 figures which provide annual sales projections to 1993 in both unit and dollar volume for each market segment as well as historical sales figures from 1985 to 1988.

The tables also provide corporate market shares for each segment including unit sales.

A separate section profiles 15 of the major competitors. This section includes background information on the company, unit sales, distribution methods, technological advancements, marketing strategy and corporate outlook.

This study is the result of primary research with emphasis on new developments, trade regulations and future trends. Report No. 921, November 1989. 112 pages, 53 tables, 22 figures, US$995.

HIV and AIDS diagnostics

This report investigates commercial aspects of AIDS screening and diagnostic testing in the US, Japan and Western Europe (Federal Republic of Germany, France and Italy), as well as Australia, New Zealand, South Korea, China, the Middle East and South America.

The report gives market share held by companies in the US and Western Europe, evaluates the companies marketing different types of tests - ELISA, confirmatory, and diagnostic screening. The competitive intelligence section of the report discusses 29 companies in depth.

Among the topics covered in the report are market size and share data, growth forecasts in units and dollars, pricing information, penetration levels, future trends and developments.

This study is the result of primary research and extensive secondary research. Among those interviewed were corporate executives, scientific and research groups, and governmental and regulatory organizations. Report No. 983, October 1989. 107 pages, 32 tables, US$795.

Diagnostics for plant diseases, pesticide residues and food. An international market analysis

This new report from Theta is an international analysis of the markets for plant diseases, food testing and pesticide residues. The primary focus of the report is on commercial aspects and related issues concerning diagnostics. The report reviews efforts being made by different companies world wide to develop applications for detecting residues and bacteria in food, for diagnosing plant diseases and for detecting and monitoring pesticide residues.

The markets for diagnostics, by application and by consumption, are discussed separately for the US, Western Europe and Japan. The report provides market data at the regional level for each of the different types of diagnostics.

The report evaluates new technologies, the role of regulatory agencies and trade regulations. Emphasis is placed on demand expectations and the receptiveness of different market regions to new technologies and resulting product applications.

This report is based on interviews with company executives, industry consultants, scientific and research groups and authorities at national, governmental and regulatory agencies. Report No. 989, January 1990. 85 pages, 54 tables, 39 corporate profiles, US$795.

Biotechnology instrumentation markets

This report deals with biotechnology and the sale of instruments to 500 companies and an additional 500 or so companies in related areas as well as 1,000 major university and public research institutions. Four sectors are addressed: companies whose main character is biotechnology; biotechnology operations within pharmaceutical companies; academic biotechnology units; and institutional biotechnology units. Products covered include: DNA synthesizers, DNA sequencers, peptide synthesizers, peptide sequencers, electrophoresis, liquid chromatography and bioreactors.

According to Theta findings, companies can expect to encounter unusual difficulty in establishing new instrument technology in this market. Currently, almost 250 biotechnology companies are competing for capital in an investment market expected to show little short term growth. Theta estimates, in the next five years, about 60 biopharmaceuticals will enter the market at a cost of US$6 billion for commercialization.

The first companies to bring products to market are generating significant sales in relatively short periods of time. Companies are learning they have to add value to their products by developing a niche market mentality.

The impact or the market of cost reduction measures, mergers, contract production activity and FDA Good Manufacturing Practices are analysed. Based on the number of products moving down the introduction pipeline, Theta believes the biotechnology instrumentation business has great cause for optimism. Sales for all categories combined are growing at an average rate of 15 per cent per year reaching US$746 million in 1993.

Each instrument category is discussed from both a technical and marketing perspective. Detailed tables provide sales by market segment for 1989 in dollars and by per cent. Other tables project growth in dollars and units by category through 1993. Five companies are profiled in a separate corporate intelligence section. Report No. 903, January 1990. 109 pages, 32 tables, US$795.

Fermentation systems

This report covers the research and pilot plant market for fermentation systems designed for micro-organism cultivation.

Companies profiled are the following: B. Braun Melsungen (B. Braun Biotech), Chemap AG, ICI Pharmaceuticals, LSL Group, New Brunswick Scientific, Porton International, Sulzer Biotech

Systems, Wheaton Instruments, and Setric Genie Industrie1. Report No. 971, January 1990. 104 pages, 27 tables, US$795.

G. MEETINGS

1990

July 1990

30 July to 2 August Kuala Lumpur, Malaysia	Trends in Biotechnology in Asia and the Pacific Region. Further details from The Secretariat, Symposium on Trends in Biotechnology in the Asia-Pacific Region, Department of Biotechnology, Faculty of Food Science and Biotechnology, Universiti Pertanian Malaysia, 43400 UPM Serdang, Selangor, Malaysia.

August 1990

15-16 August Spokane, Washington, USA	AgBiotech International: Business Opportunities for the 90s. Further details from AG Bureau, P.O. Box 2147, Spokane, Washington 99210, USA.

September 1990

2-5 September Paris, France	Institut Pasteur. International Conference on Bacillus subtilis Genome. Further details from the Secretariat of the Conference. Unité de Biochimie Microbienne, Institut Pasteur, 24 rue du Dr. Roux, 75724 Paris Cedex 15, France.
4-10 September Cold Spring Harbor, New York, USA	Origins of Human Cancer. Further details from the Meetings Co-ordinator, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724, USA.
6 September Paris, France	Institut Pasteur, Paris, France. Amersham Symposium on the Technological Aspects of Mapping and Sequencing Genomes. Further details from the Symposium Secretariat, Institut Pasteur, 25 rue du Dr. Roux, 75724 Paris Cedex 15, France.
7-8 September Cambridge, UK	Fourth Cambridge Symposium on The Neuroiogical Basis of Anxiety. Further details from The Symposium Secretary, Parke-Davis Research Unit, Addenbrookes Hospital Site, Hills Road, Cambridge CB2 2QB, UK.
7-9 September Aberystwith, Wales, UK	University College. Fifth Harden Discussion Meeting on Bio-chemistry and Physiology of Inorganic Phosphate. Further details from Dr. S.P. Shirazi-Beechey (Harden Discussion Meeting), Department of Biochemistry, University College of Wales, Penglais, Aberystwyth, Dyfed SY23 3DD, UK.
17-20 September St. Louis, Missouri, USA	AgTechnology '90. International Conference and Seminar on Agriculture - The Decade Ahead. Further details from Bill Freiberg, AgTechnology '90, P.O. Box 7, Cedar Falls, IA 50613, USA
24-27 September Cold Spring Harbor, New York, USA	Centennial Symposium on Evolution: From Molecules to Culture. Further details from the Meetings Co-ordinator, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724, USA.
26-28 September Piscataway, New Jersey, USA	Advances in Receptor-ligand Interactions. Further details from Advances, Center for Advanced Biotechnology and Medicine, 679 Hoes Lane, Piscataway, NJ 08854-5638, USA
27-28 September Rennes, France	Polyamines: Chemistry, Biology and Medicine. Further details from the Department of Cell Biology, Faculty of Medicine, 2, ave. du Prof. Leon Bernard, F-35043 Rennes, Cedex (France)

October 1990

11-12 October New York, USA	The Plaza Hotel, New York, USA. Immunology in the 21st Century. Further details from the Registration Manager, SLACK, Inc., 6900 Grove Road, Thorofare, NJ 08086, USA.
24-26 October Adelaide, Australia	IMVS Second Research Symposium on Cancer Research and Treatment - Directions for the 21st Century. Further details from Prof. M. Vadas, IMVS, P.O. Box 14, Rundle Mall, Adelaide, S. Australia 5000, Australia.
30 October to 1 November Helsinki, Finland	KEMIA '90 - Finnish Chemical Congress - Symposium on Biotechnology (Forest Industry) Further details from Ms. Eeva Kota-aho, Association of Finnish Chemical Societies, Hietaniemenkatu 2, SF-00100 Helsinki, Finland.

November 1990

7-14 November Alfriston, East Sussex, UK	International Science and Technology Forum on Environmental Policy and Management. Further details from British Council Representatives overseas or from the Courses Department, The British Council, 65 Davies Street, London W1Y 2AA, UK.
27-29 November Washington, DC, USA	BIOTECH USA. Further details from Ms. Gina Amatruda, CMC/BIOTECH USA, 200 Connecticut Avenue, Norwalk, CT 06856-4900, USA.

December 1990

9-12 December Tsukuba, Japan	Second International Conference on Endothelin. Further details from Dr. Katsutoshi Goto, Department of Pharmacology, Institute of Basic Medical Sciences, University of Tsukuba, Tsukuba, Ibaraki 305, Japan

1991

April 1991

22-24 April Florence, Italy	Congress Palace, Florence, Italy. International Symposium on Hereditary Tumours. Further details from Dr. Maria L. Brandi, Department of Clinical Physiopathology, University of Florence, Viale Pieraccini 6, 50139 Florence, Italy

June 1991

9-15 June Frankfurt-am-Main, FRG	ACHEMA'91. International Meeting on Chemical Engineering and Biotechnology. Further details from DECHEMA, P.O. Box 97 01 46, Theodor-Heuss-Allee 25, D-6000 Frankfurt-am-Main 97, FRG

July 1991

28-31 July Oxford, UK.	B.t., '91. Meeting encompassing all aspects of current work on Bacillus thuringiensis. Further details from Dr. Phillip F. Entwistle, Institute of Virology and Environmental Microbiology, N.E.R.C., Mansfield Road, Oxford OX1 3SR, UK.

H. SPECIAL ARTICLE

THE CONCEPTS OF RISK ASSESSMENT*

by

Alvin G. Lazen**

This paper discusses the concept of risk assessment, how it is addressed in different sets of regulations and guidelines, and what methods are most practicable. Several authors have written about regulatory matters, but here emphasis is placed on the science of risk assessment. The paper will also focus on the concepts, or philosophy underlying risk assessment and will attempt to fulfil those goals by discussing risk assessment and management examples of risk assessment methods and a "practical" risk assessment method.

Initially, readers may be reminded of the distinction between risk assessment and risk management. In the area covered here, risk assessment of genetically engineered organisms, the two realms are mixed as a matter of course. Discussions on genetic engineering are consumed by the means to regulate them. That is a risk management decision, a value judgement, that should begin, ideally, only after a scientific assessment of potential risk has been performed. The word "ideally" must be accorded its full importance because in genetic engineering as well as older and better understood situations of chemical risk analysis and human health risk assessment, it is not realistic to hope to completely separate the scientific assessment from value judgements.

The paper will then briefly discuss the variety of assessment regimens for ecological risks associated with the release of genetically modified organisms, mainly as they have evolved in the United States.

In passing, a comparison of the US approaches to other countries' approaches to the assessment of ecological risks will be given.

In conclusion, an outline of the components of a practical scheme for assessing risks will be covered, in speculation of how this "practical" scheme may be applied in developing countries.

Risk assessment and risk management

Human health risk assessors have defined the four steps in risk assessment of human health hazards as: hazard identification, dose-response assessment, exposure assessment, and risk characterization. In other words, determining the probability of harm attributable to exposure to a particular agent and, to the extent possible, quantifying the magnitude of risk. These are matters based on scientific information. Although the terms do not apply exactly when considering ecological risk assesment, the concepts behind them do, that is, we wish to determine the probability of harm to the environment and the possible extent of that harm.

In contrast, risk management is the political, economic and social business of determining what to do about the risk. For example, if a scientific risk assessment determines that exposure to asbestos is a hazard, should society continue its use, tear out the asbestos insulation from every building, ban the future use of it but leave it in present structures, or find some other means of managing the situation?

An example from genetic engineering is that we may scientifically determine that a gene has been introduced into a plant that makes it resistant to an economically important insect pest. However, there is a chance, in this hypothetical situation, that the gene may be transferred to a weedy relative if the weedy relative is in the vicinity and impart pest resistance to the weed. So much for science. It is then a risk management decision to determine whether to permit that engineered plant to be propagated in a field trial, or more widely on a commercial basis, and under what conditions, or not to be released at all. Scientific considerations end at the point where scientists have assessed the degree of risk of introducing the modified plant into the environment; management considerations begin in deciding whether the risk is small enough to endure or, a different and harder decision, whether the benefits outweigh the risk.

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Many of the trains of thought and decisions being labelled "risk assessment" for genetically modified organisms are value judgements with little reference to the science of risk assessment. For example, the decision to regulate organisms made by the process of genetic engineering rather than products on their own merits is a societal value judgement that genetic engineering is a special case and an a priori assumption that the products are hazardous. The generally accepted practice of examining each proposed release on a case-by-case basis is also an expression of the above assumption. It would be as easy for those who have established this practice to have decided instead that a scientific determination will be made of "low-risk" categories of releases and that when a proposed release fits into this category, it will not be scrutinized in detail before approval.

Think for a moment about the many meetings and reports on genetically modified organisms. Almost all deal with how to regulate them and not on how to assess the risks. To be sure, almost all of these discussions and reports start with the scientific elements that must be considered when making the regulatory decision. However, how many can you think of that focus only on the scientific questions that must be asked and answered to determine the potential hazard of the use of a genetically engineered product? With apologies to those readers who know the world-wide practices better than I, and with the admission that I may not have relevant information from other parts of the world available to me, let me say that it is in the United States that one may find more examples of efforts to examine and analyse scientific risk assessment in detail.

I will present to you some of the ways risk assessment has been approached. Most of my examples are drawn from work in the United States.

Concepts of risk assessment in the United States

Others have detailed the regulatory regimens for assessing and managing the risks of releasing genetically-engineered organisms. I will review briefly those elements that are background to my focus on the assessment of the safty or risk of organisms.

Decisions about releasing genetically-engineered organisms had first been made (until the early 1980s) by the Recombinant Advisory Committee of the National Institutes of Health. NIH/RAC has no regulatory authority but their rulings were followed on a voluntary basis. Please note that the assessment of risk was carried out by a scientific organization and not in a regulatory milieu. After several decisions by NIH were challenged in court under the National Environmental Protection Act on the grounds that environmental concerns were not being taken into account by an organization that is known for its human health expertise, NIH/RAC has worked with the USDA and EPA in helping to make decisions on environmental releases but has not acted unilaterally.

Now, under a "Co-ordinated Framework" published in June 1986, the responsibility for assessment is shared mainly by three agencies: the Environmental Protection Agency, the Department of Agriculture, and the Food and Drug Administration. Briefly stated, the Food and Drug Administration is responsible for human diagnostic and therapeutic products, for food products derived from genetic engineering techniques (with USDA/FSIS), and for animal drugs. The Department of Agriculture assesses and regulates animal vaccines, field tests of genetically-engineered plants and agriculturally-relevant micro-organisms (APHIS together with the Environmental Protection Agency). The Environmental Protection Agency is responsible for assessing and regulating the use of certain "new" micro-organisms in the environment and, with the USDA, as mentioned above, for plants.

The means by which these agencies assess the risks of genetically-engineered organisms is hard to describe in any brief way because the scientific approaches vary and the assessments are often constrained or tailored to fit the law that governs a particular agency or the office within an agency. For example, EPA invokes the Toxic Substances Control Act in regulating new organisms and, since that law applies to new chemical agents, they have defined recombined DNA as a "new chemical".

Several characteristics are shared in common among the agencies in the way they assess the risks. Two use advisory committees to help them decide on science and science policy matters. These are the EPA's Biotechnology Science Advisory Committee (BSAC) and the USDA's Agriculture Biotechnology Research Advisory Committee (ABRAC). All make decisions on a case-by-case basis, i.e., there is no general exemption of classes of products. And, though this may change, all tend to make decisions on the basis of process (that is that DNA biotechnology was used) rather than on scrutiny alone of the derived product.

The scientific data requirements of the agencies also share commonalities. When DNA has been moved from one organism to another, the agencies require information about the source organism, the identity and characteristics of the DNA transferred, and the mechanism by which it was transferred. The agencies then require information about the derived organism, including data on the characertistics expressed before and after manipulation, such as the likelihood of competitive success in the environments and of subsequent genetic transfer to other organisms. Usually, they require data on the characteristics of the planned introduction such as the environment into which the organisms will be released, the size of the release area, and the number of organisms to be introduced.

The collected data is reviewed by the agencies applying scientific principles to the determination of risk. Before describing how the agencies do that, I will refer to the development of the lists of scientific principles and frameworks for consideration of decision-making principles.

Numerous meetings and reports in the late 1970s and 1980s dealt with the scientific considerations for deciding on the possible ecological impact of introducing genetically modified organisms into the environment. From these there evolved a consistent set of scientific issues that must be considered. In its broadest categorization, these may be separated into genetic considerations and ecological considerations. In somewhat more detail, the issues were separated into those summarized in figure 3.

Identifying the questions that must be asked and answered is critically important without doubt. However, even more important is the framework in which these questions are asked and answered. It is within a framework that priorities may be set on the importance of the individual questions. And it is when an orderly framework is used, that a logical march towards a decision may be realized. Some of the ways into which the general scientific issues

mentioned above have been organized are described hereunder. These have all been published in the very recent past in a paper prepared by a committee of the Ecological Society of America in April (Tiedje et al., 1989). Here, the four categories of risk assessment information (attributes of genetic modification, e.g. deletion; attributes of the parent organism, e.g. level of domestication; phenotypic attributes of the derived organism compared to the parent, e.g. fitness; and attributes of the environment, e.g. proximity of weedy relatives) are further detailed. The issue is not only mentioned but a scale of the level of scientific consideration to be accorded the issue is included. In a footnote the authors caution that they consider the scales to be quantitative or semi-qualitative and that the level of consideration depends upon the relationship of one issue to another.

Attention is drawn to the fact that the approach deals with scientific issues only. No risk management considerations enter into it.

A paper from the Cornell University Ecosystem Research Center (Gillett et al., 1985) is an example of another form of organizing the questions into a framework.

An added feature of this structure is that it lists the questions and data needs together under a particular category.

Let us look now at an approach to arranging many of these issues in a "decision tree" framework for decision-making. A draft paper by Beak Consultants Limited for the Canadian Government (Major et al., August 1988) to serve as background to the development of guidelines for regulation of biotechnology products under Canada's Pest Control Products Act and the Fertilizers Act depicts an ordering of the questions to be asked, a "decision tree" approach where a "yes" or "no" answer to a particular question shunts the user to the next set of questions, to a conclusion, or refers the reader to the relevant regulatory provisions.

I will now discuss the concepts and approach proposed in a report that is close to my heart because I was involved in helping a committee of the US National Academy of Sciences/National Research Council prepare the report published in September 1989.

It is important first of all to understand the conceptual framework in which the committee formulated its ideas. First, that one must judge the safety of a product strictly on the basis of that product's characteristics, not on whether the product derives from a classic process or a modern genetic process. This is in line with general practices in other fields. One does not judge the safety of an automobile on the basis of whether it is individually hand-made, assembly-line made, or put together by computer-driven robot machines. One wants to know if its brakes stop the car, its throttle works properly, and its lights go on when they are supposed to. Second, the committee, on strictly scientific grounds, viewed a genetically-engineered organism to be no different, in principle, than other living things. In every case, recombinations occur, genes are transferred, and one must test in the greenhouse or field to learn if one has obtained a desirable or undesirable product. Farmers and scientists have been doing this for a long time, first intuitively and then scientifically. Genetic crosses are made, various plants grow, the good are propagated and the bad are discarded.

There appears to be some misunderstanding of the report's reliance on product rather than process. The committee stated that process should be used as a means to understand the product. Some have interpreted this to mean that this report, also, relies on process. It does not.

Based upon these concepts, the committee proceeded to develop a framework for decision-making. I believe this framework is scientifically sound, reasonable and easy to understand and could perhaps be used in developing a complete regulatory regimen. What it is not, however, is detailed. It is a conceptual framework that does not list every question that may be asked and needing answers.

Let us examine the conceptual elements of the framework.

One first determines whether there is reasonable assurance that the organism and other conditions of an introduction are essentially similar to known introductions and that these have been proven to present negligible risk. Use of the familiarity criterion allows assessors to draw on past experience with such introductions and provides flexibility in that as additional experience is gained, future decisions can be made more easily. It permits the establishing of low-risk categories and of making decisions on a categorical rather than on a case-by-case basis.

Familiarity does not necessarily mean safe. Rather, to be familiar with the elements of an introduction means to have enough information to be able to judge the introduction's safety or risk.

If knowledge of the type of genetic modification, the species being modified, or the target environment is insufficient to meet the familiarity criteria, the proposed modification must be evaluated with respect to the ability to control or confine the introduced organism and to the potential effects of a failure to confine or control it. The results of these latter evaluations will define the relative safety or risk of a proposed introduction.

The framework for decisions presented by the NAS/NRC committee is a decision-tree approach using examples of the scientific questions to be asked. The organization is on the basis of the conceptual framework described.

My next example of a framework for decision-making is one developed by the US Department of Agriculture (USDA, 1989) and shows a formal approach for categorizing risk. The document is a draft and is not in use by Department staff. Note that this process is used for determining the risk or safety of releases that are small and for research.

The USDA's stepwise evaluation process shows that the first three and a half steps are clearly scientific risk assessment. These are: determining the safety category of the unmodified organism, the type of modification, and the safety category of the unmodified organism. Step 4 is to identify the confinement level needed in consideration of the assessment performed in steps 1 through 3. Step 5 is the administrative review at other levels of the USDA and approval.

Two other examples show the evaluation factors used to determine the safety category of the unmodified organism. First, the evaluation factors related to the characteristics of the organism per se, such as pathogenicity, infectivity, etc., and

next how the categories in which the unmodified organism would be placed depending on its potential for adverse effects on health or the environment.

Another shows examples of organisms displayed by the expected safety category. The list proceeds from corn, etc., in the highest category of safety, and foot and mouth disease virus, etc., in the lowest.

Examples of types of genetic modification are also presented. From this information a safety category is determined for the modified organism. We now have a safety classification for the parent organism, knowledge of the kind of genetic modification performed and a safety classification of the modified organism. There remains then the need to determine an appropriate confinement classification, the fourth step in the USDA process.

The EPA similarly has staff review data but often uses an outside (in the sense of non-EPA employees) advisory committee to assist in analysing the data. The EPA has not published a chart or similar document to those previously presented because of debate about drafts that have been shared among some communities. It has made and continues to make its decisions based upon its interpretation of the Toxic Substances Control Act and the Federal Insecticide, Fungicide, and Rodenticide Act and a set of working definitions relevant to genetically modified organisms. Following these laws it has felt constrained to identify Recombinant DNA as a new chemical and to separate by definition those genetically-altered organisms that need not be assessed or regulated because they were modified by a classical technique, e.g., UV irradiation or spontaneous mutation, or by a modern molecular manipulative technique. Further, they give special scrutiny to those derived organisms that have received DNA from an organism phylogenetically distant from the derived organism.

Especially in the EPA's case and to a far lesser extent in the USDA, the process by which an organism has been made is considered highly important. This is in response to the perception of special risks of genetically-engineered organisms by the public that ultimately sets the agenda for the agencies.

What are the practical means for assessing risks?

The following is a list of some elements of a "practical" means for assessing risk. It is necessary first to know what one means by "practical". Does the term imply practical in the sense that it gives a valid scientific answer to the question: how much risk exists? Does it mean that it is practical in a regulatory sense, i.e., fits the existing laws? Does it mean practical in informing, including, and achieving the consent of the public that lives in the vicinity of a proposed release? For my purposes, I will adhere to the definition of practicality that asks, "What are the practical means for determining on scientific grounds alone the risks of an introduction". In my own mind, I think of the terms "desirable" or "ideal" rather than "practical".

- "Practical" risk assessment
- Science based
- In agency responsible for regulated product
- Co-ordinating body
- Assess all products
- Use expert panels
- Panels to do scientific assessment free of regulatory concerns
- Use categories of risk
- At local level where possible
- Use scientific issues lists
- Genetics and ecology included in assessment
- Consider geography

We have cited examples of lists of issues to be considered in assessing risk, frameworks that are conceptual such as the NAS/NRC one, and frameworks that are detailed and can be directly related to regulation such as those of the Canadian consultants and the USDA classification scheme. Which is the practical one? My opinion is that none of these is perfect but almost all have components that would be useful. I will list what I believe are the elements of a practical (or desirable) system for assessing the risks.

The first element is that the system should be strictly science-based. A science-based system would emphasize assessment of a product and not the process by which the product arose. The system would not label products of genetic engineering as a special case of hazard but as part of the biological continuum of phenomena that occur when genes are exchanged. A science-based system would not allow risk management decisions to be mixed in prematurely.

One of the reasons I believe no perfect risk assessment system has yet been described is that most still rest to a lesser or greater degree on considerations of the process of genetic engineering rather than judging products on their merits.

Because the system is based on the scientific assessment of the products, and regulatory responsibility is usually assigned on the basis of products (drugs to a drug agency), the risk assessment should be managed within that agency. That agency should have the best ability to identify the experts in their area who can assess the risk.

An umbrella organization is needed to perform the functions that will co-ordinate the efforts of the individual agencies such as ensuring that all agencies use the same definitions, that overlap in responsibilities is understood, and that assessments are performed with equal and complete rigour.

ALL products of genetic modification should be assessed. To include all products reduces the need for definitions that sometimes are based on "grandfather clauses" but have little basis in scientific reality. For example, grandfathering UV irradiated bacteria when, on scientific grounds, these are no more or less in need of assessment than are bacteria modified by modern molecular techniques.

Assessments must be done by the most expert scientists who represent a balance of views. When all is said and done, risk assessment is a matter of expert judgement. Experts do not always make the same judgement. For our topic, a good example is that experts disagree whether it is valid to compare the introduction into the environment of an organism modified by a few genes, to the introduction of a new and complete genetic entity (rabbits into Australia, for example). Another example of disagreement is on the question of whether an organism into which non-coding regulatory gene regions have been transferred requires lesser scrutiny than other genetically modified organisms. Thus, it is not a perfect protection against disagreement or error to have experts make the judgements. However, the chances of a correct decision rise in proportion to the expertise of those making the assessment.

The experts must be allowed to work in an environment free, to the extent possible, of the pressures and sometimes non-scientific matters that exist in the regulatory environment. They are mandated to arrive at a scientific assessment of risk. The regulators can enter the picture after that determination is made.

The practical system should aim at categorizing releases into levels of risk so that categories of releases that are exempt from case-by-case analysis can be rapidly developed and applied. The familiarity, confinement, potential risks to the environment framework of the NAS/NRC report is, I believe, a large step in the direction of identifying categories. The USDA classification scheme is an excellent example of an orderly way to use categories.

As soon as possible, and certainly when low-risk and exempt categories are established, risk assessment decisions should be made at a local level, i.e., at the institution where the work will be performed.

The system should consider all scientific issues that can contribute to a full understanding of the release conditions and result in a valid assessment. There is no need to re-invent such a list. The Ecological Society of America list is a good one. The temptation should be avoided to use such lists as check lists where absolute proof on each issue in turn must be obtained. Proof that satisfies the criteria of experts should be the goal.

Equal emphasis should be placed on genetic considerations and ecological considerations. The expertise represented on the assessment panel should be of both kinds.

Because the ecosystem varies by geographical location, the experts must take this also into account. The practical system must use persons who know the ecosystem of the country in which the release is intended to be performed.

Many of the elements described may not be adaptable to situations in countries which are less developed because of the lack of decision-making infrastructure, lack of a legislative framework to enforce decisions made or perhaps because of a lack of available expertise. Yet potentially great benefits for food and fibre production, animal husbandry, human health and environmental protection could be achieved if biotechnology solutions were applied in these countries. It is therefore important to seek means to make it possible to safely test and eventually use biotechnology in lesser developed countries. A first step is to find a means to make decisions about the safety of an introduction even when the country in which a test or application needs to be done is not in a position to make the decision. Some possible means of reaching such decisions are speculated upon below.

1. An international organization such as an appropriate unit or combination of units associated with the United Nations could organize committees of international experts to consider and act on proposals for introductions of organisms into the lesser developed countries.

2. Countries such as the United States, the United Kingdom and Australia that have already established committees to make decisions on environmental introductions might arrange for those committees to accept proposals for introductions and provide an advisory decision to the country in which the introduction is proposed.

3. An international organization specifically charged to render decisions on environmental introductions might be formed de novo on the model of the International Agency for Research on Cancer (IARC) in Lyon, France, which considers evidence concerning cancer risks from exposure to chemical agents and publishes its findings for the international scientific community.

Because I have tried to focus on the scientific elements of a practical plan, I may leave the impression that I undervalue the importance of trans-science matters such as the public's attitude about science and government and public perception of genetic engineering. Further, "practicality" founders often on the shoals of the need to adhere to laws and regulations that do not exactly fit the situation to be regulated.

We have ample evidence that the public has the final word on whether genetically modified organisms will be released based on what they think about the safety. Not even the perfect risk assessment will convince them if there is no trust. How to gain the trust of the public is a subject for another article.

Reference and reading list

Dixon, B. 1988. Engineered Organisms in the Environment: A "lay" summary based on the First International Conference on the Release of Genetically Engineered Micro-organisms (REGEM 1) held in Cardiff, United Kingdom, 5-8 April 1988. Cardiff, United Kingdom: Qualitex Printing Limited. 47 pages.

GAO (United States General Accounting Office). 1988. Biotechnology: Managing the Risks of Field Testing Genetically Engineered Organisms. GAO/RCED-88-27. Washington, DC; Government Printing Office. 108 pages.

Gillett, J. W., Stern, A. M., Levin, S. A., Harwell, M. A., Alexander, M., and Andow, D. A. 1985. Potential Impacts of Environmental Release of Bictechnology Products: Assessment, Regulation, and Research Needs. Ithaca, NY; Ecosystems Research Center, Cornell University, ERC-075. 219 pages.

Major, D. W., Hart, D. R., and Lush, D. L. (Beak Consultants Limited). 1988. Release of Genetically Engineered Micro-organisms into the Environment. Ottawa, Canada. An unofficial working paper prepared under contract to the Pesticides Directorate and Feed and Fertilizer Division of the Food Production and Inspection Branch, Agriculture Canada. 43 pages.

NAS (United States National Academy of Sciences). 1989. Field Testing Genetically Modified Organisms: Framework for Decisions. Washington, DC; National Academy Press. 170 pages.

NAS (United States National Academy of Sciences). 1983. Risk Assessment in the Federal Government: Managing the Process. Washington, DC; National Academy Press. 191 pages.

OECD (Organisation for Economic Co-operation and Development). 1989. Good Development Practices for Small-Scale Field Research with Genetically Modified Plants and Micro-organisms. (An unofficial working draft of a document in preparation.)

OTA (United States Office of Technology Assessment) US Congress. 1988. New Developments in Biotechnology, Field-Testing Engineered Organisms: Genetic and Ecological Issues. OTA-BA-350. Washington, DC: Government Printing Office. 150 pages.

Royal Commission on Environmental Pollution. 1989. The Release of Genetically Engineered Organisms to the Environment. London, UK: Her Majesty's Stationery Office. 144 pages.

Tiedje, J. M., Colwell, R. K., Grossman, Y. L., Hodson, R. E., Lenski, R. E., Mack, R. N., and Regal, P. J. 1989. The Planned Introduction of Genetically Engineered Organisms: Ecological Considerations and Recommendations. Ecology 70:298-315.

USDA (United States Department of Agriculture). 1989. USDA Guidelines for Research with Genetically Modified Organisms Outside Contained Facilities. Washington, DC; Office of Agricultural Biotechnology. 24 pages.

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